7-1-96

MIDI Prep of Plasmid DNA from Transformed DH1

1) culture started from colony w/ 100 µl cells (see previous) plated @ 3 ml → then 30 ml
2) MIDI Prep of plasmid DNA (see Qiagen MIDI Prep protocol)
3) DNA resuspended in ~30 µl TE

Notes: * culturing of DH1 was very slow; about 8hr. to get from clear media to turbid
* centrifugation to pellet DNA during MIDI prep was not favorable; DNA not readily visible so DNA ~~easy~~ (or sufficient quantities of DNA) may not have been isolated

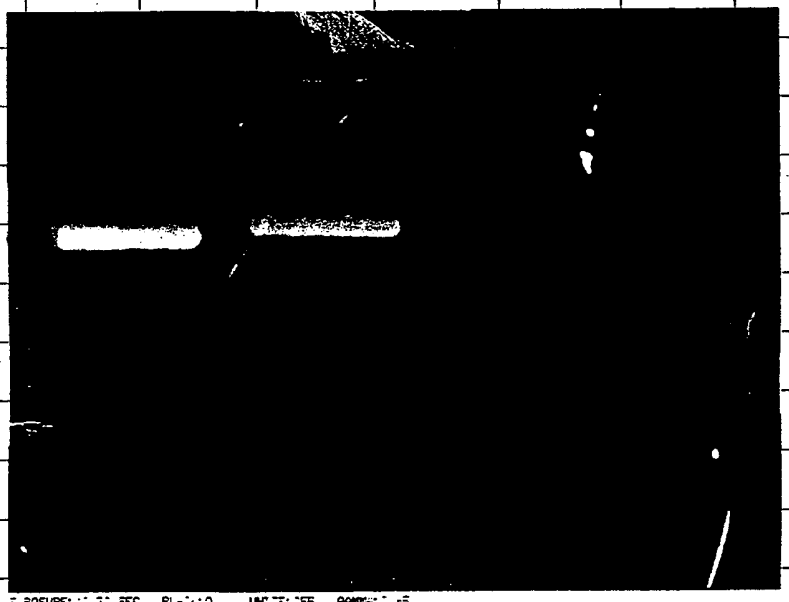
7-1-96 → 7-2-96

Digestion of pEGFP-N w/ Age-I & Bsp EI and pFOXSB w/ Bsp EI

total rxn volumes 100 µl

7-2-96

1) pEGFP-N (~~30~~ µg (30 µl from previous experiment) was digested w/ Age I @ room temp. overnight (4 µl enzyme)
2) DNA was reprecipitated w/ cold EtOH, resuspended and cut w/ Bsp EI (4 µl) for ~4 hr. @ 37° C
3) Concurrently, pFOXSB (5 µg) was cut w/ Bsp EI (4 µl) and incubated @ 37° for ~4 hr.
   * pEGFP-N (cut w/ Bsp EI) and pFOXSB was digested in 50 µl total volume rxn.
4) Digested pFOXSB was then mixed w/ CIP for ~~20~~ 25 minutes
5) Samples run on 1.5% agarose gel (100 V) w/ λ marker



* used only ½ amt. of EtBr in agarose

	pFOXSB	pEGFP-N	λ
cut w/:	BspEI	BspEI + AgeI	

7-2-96

6) pFOXSB (BspEI cut) band and pEGFP-N 750 bp band was cut out from gel and purified using Qiagen Gel Extraction kit
* pFOXSB band = 215 mg
* pEGFP-N 750 bp band = 206 mg
7) DNA eluted from spin column was resuspended in 50 µl 1x TE

Ligation of pFOXSB and pEGFP-N 750 bp fragment (EGFP + NLS)

1) The following ligation reactions were setup:

Tube #	contents
1	1 µl cut pFOXSB + 5 µl pEGFP-N fragment + 1 µl ligase + 1 µl ligase buffer + 2 µl ddH_2O (ligase added last) tot. vol = 10 µl
2	1 µl cut pFOXSB + 10 µl pEGFP-N fragment + 1 µl ligase + 1.5 µl buffer + 1.5 µl ddH_2O tot. vol = 15 µl
(control) 3	1 µl cut pFOXSB + 9 µl ddH_2O (for background) tot. vol = 10 µl

2) the above contents were mixed (except for ligase) in Eppi & centrifuged briefly; then ligase was added

Ligation (cont'd) 7-2-96

3) ligation rxn. incubated overnight @ 14-16°C

7-3-96

Plasmid MIDI Prep

1) MIDI Prep performed on DM1 transformed cells from a second culture (prepared by Maike) following culture procedures described in Qiagen plasmid MIDI protocol. (see 7-1-96)
   * DM1 culture yielded more cells this time (~3X)

* pellet bacteria from culture by spinning @ 4° for 10' speed 4

2) for MIDI prep., P1 buffer and P2 buffers were added before resuspension ~~and mixing~~ of cells.

3) After ~~5' incubation in ice~~ the addition of P3 buffer and subsequent incubation on ice for 15', the mixture was aliquoted into Eppi's w/ 1 ml in each tube for a total of 11 tubes.

4) The tubes were then centrifuged @ hi speed for 30' in microcentrifuge (@ RT (room temp.))

5) Qiagen tip-100 was equilibrated w/ 4 ml QBT buffer

6) Supernatant from each tube was applied to column

7) Column washed 2x10 ml QC buffer

8) DNA eluted from column w/ QF buffer (5 ml) and collected in Eppi's (aliquoted to 500 µl per tube)

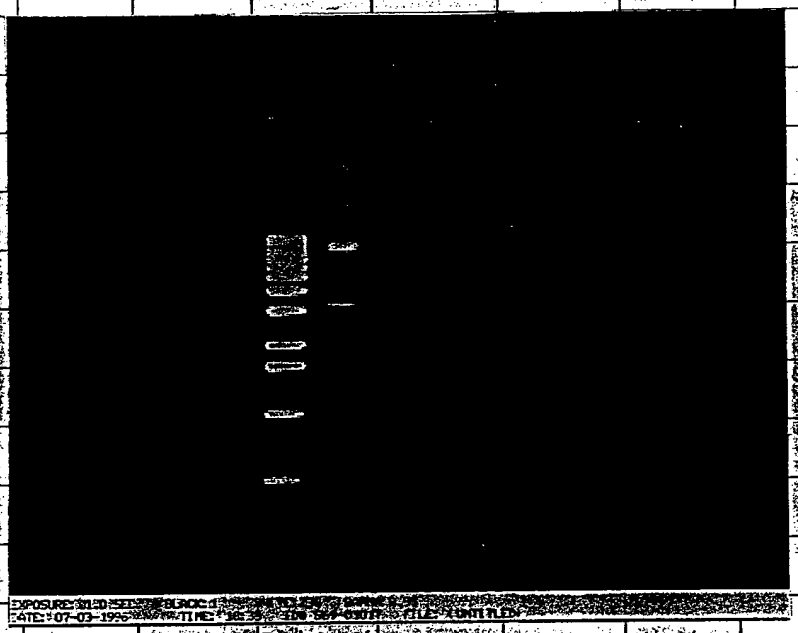
9) DNA was ppt. by adding 350 µl isopropanol to each tube & centrifuging @ hi speed (RT) for 30'

Plasmid MIDI Prep (cont'd) 7-3-96

10) Ethanol (cold & 70%) was used to wash DNA (500 µl 70% / tube) and redissolved in 75 µl TE 1X
* 75 µl TE was added to tube 1 and this same TE was used to resuspend ppt. DNA in the rest of the 9 tubes; ultimately, all DNA (originally separated in 10 tubes) is resuspended in 1 tube labelled "pEGFP-N (MIDI) 7-3-96"

run @ 100V

11) an aliquot of this prep was then run on an agarose (1%) gel:



* 10 µl λ marker
* pEGFP-N1 & pGK-neo = 1 µl plasmid

7-3-96

Transformation of Competent cells w/ pFOXEGFP-N1 ligation

*bacteria used is HB101

1) cells thawed on ice (100µl cells/tube), then add all of ligation rxns. to tubes of cells
2) a fourth transformation rxn. was performed using BS ΔNot to transform cells (1µl BS ΔNot) for Maike
3) incubate on ice for 30'; warm 0.9ml LB @ 42°
4) heat shock @ 42° for 45 sec and ice for 2'; then add ligation rxns. to ~~cells~~ LB (all of the rxn. tube)
5) incubate @ 37° for 1 hr.; briefly spin down cells (5 sec.) & remove 900µl & resuspend cells in remaining 100µl media
6) plate on amp plates as follows:
   a) plate 100µl of 5µl insert, 10µl insert and control
   b) streak plate w/ loop 5µl BS Δ Not
   * 1µl BS ΔNot → 10µl total vol. w/ ddH$_2$O; then added to 100µl competent cells
7) incubate @ 37° ~~for 1hr (2)~~ overnight
8) picked 24 colonies from plate w/ 10µl insert and grown in culture tubes w/ 3ml LB overnight in shaker

D

7-5-96

Miniprep of pFOXEGFP-N1 and Endonuclease Digestion

1) put 1 ml of liquid culture (from total of 24 cultures) in Eppi's and spin 50 sec.
2) pour off supernatant by ~~flicking~~ inverting tube once; this leaves ~50 µl in tube; resuspend pellet in remaining media by vortexing
3) add 300 µl TENS (cell lysing) mix and 150 µl 3M NaOAc pH 5.2 mix; centrifuged @ hi speed for 3'
4) supernatant transfered to new Eppi's and added 900 µl 100% cold EtOH; mix by inverting tubes; then, centrifuge @ hi speed for 3'
5) aspirate off supernatant and wash w/ cold 70% EtOH; ~~gently invert tubes~~ and centrifuge @ hi speed for 5' (do not mix or invert tubes!)
6) aspirate off supernatant and resuspended in 30 µl 1x TE

Digestion w/ EcoRI

1) digestion of pFOXEGFP-N1 w/ EcoRI were setup as follows:

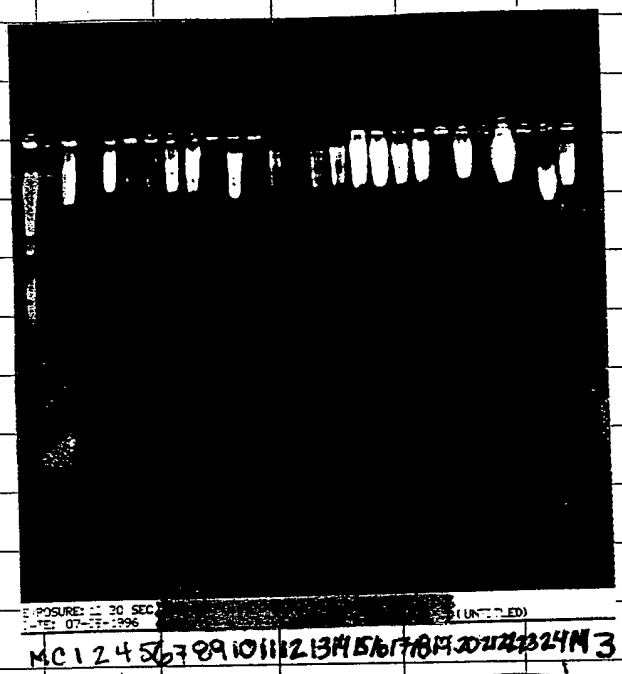
5 µl DNA (pFOXEGPN-N1; ~~and~~ pFOXSB as control)

master mix prepared on ice:
- 0.1 µl RNase (10 mg/ml)
- 0.5 µl EcoRI
- 1 µl Buffer I
- 3.4 µl ddH_2O

10 µl total volume

Digestion of pFCXEGFP-N1 (cont'd) 7-5-96

2) digestion @ 37° was performed for 2 hrs.
3) add 2 µl loading dye (6X) to each tube and load samples on 1.5 % agarose gel and ran @ 90 V :

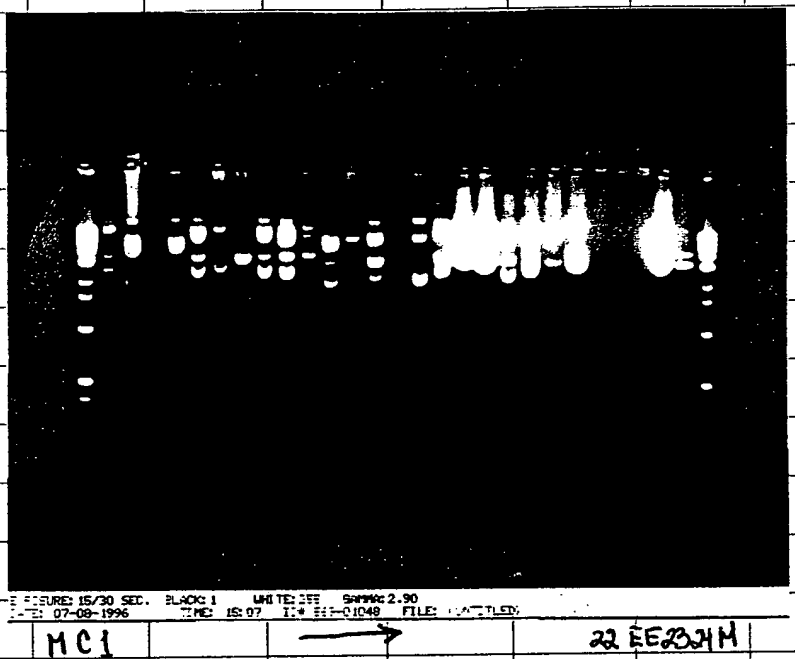


* 2 and 13 = no DNA
* something wrong w/ gel ?
* DNA does not appear digested well ; digest > 2hr ?
* not well digested b/c used Buffer 1 ?
* control = pFCXSS (1 µl plasmid)

Redigestion of pFOXEGFP-N1 miniprep 7-8-96

1) digestion rxns. were setup as previously described except used EcoRI buffer (1x in sample). and only ~ 0.5 µl pFOXSB as control (ran out of pFOXSB)

2) digested @ 37° for ~2 hrs. 20 min and loaded onto 1.5% gel @ 100 V:



* 175 band not seen; low molar concentration?!

* loaded 20µl 1x marker
* control = ~0.5 µl pFOXSB cut w/ EcoRI (12µl)
* all spl loaded were ≥ 12 µl
* lanes between 22 & 23 were empty
* presence of 850 bp band in 24 but no 175 bp band is observed for all spl; this may have resulted from incomplete digestion and dirty prep
* will make a new prep of colony 24 and redigest

7-8-96

Reculture of colony 24 for miniprep

1) put sterile LB (3 ml) into 2 culture tubes each (LB + amp)
2) flame an inoculating loop, cool and dip into tube w/ colony 24 and dip into fresh tube w/ LB to reculture; repeat a second time and incubate tubes @ 37° overnight until turbid;
additional tubes (for Maike):
   ⓐ BS Δ Not
   ⓑ pGK-neo
   * for these, inoculated a colony from each plate and cultured overnight in 3 ml LB tam;

Miniprep (Qiagen) of colony 24 ⓐ BSΔNot ⓑ pGK-neo 7-9-96

* bacteria used is HB101

① pellet bacteria by spinning (speed 4) @ 4° C for 10'; discard supernatant
② add 300 µl Buffer P1 and resuspend pellet; transfer to Eppi's
③ add 300 µl Buffer P2 gently mix and incubate @ RT for 5'
④ add chilled Buffer P3 (300 µl); invert 5-6 x and incubate on ice for 10'
⑤ centrifuged @ hi speed for 15'; supernatant moved to 20-tip column (equilibrated)
⑥ wash 4 x 1 ml Buffer QC, then DNA eluted w/ 800 µl Buffer QF

Miniprep (cont'd) 7-9-96

⑦ DNA ppt. w/ 560 µl isopropanol (0.7 vol) and centrifuged @ hi speed for 30'

⑧ DNA washed w/ 1 ml 70% cold EtOH, centrifuge 15 sec. and redissolved in 20 µl 1X TE

Digestion of Colony 24

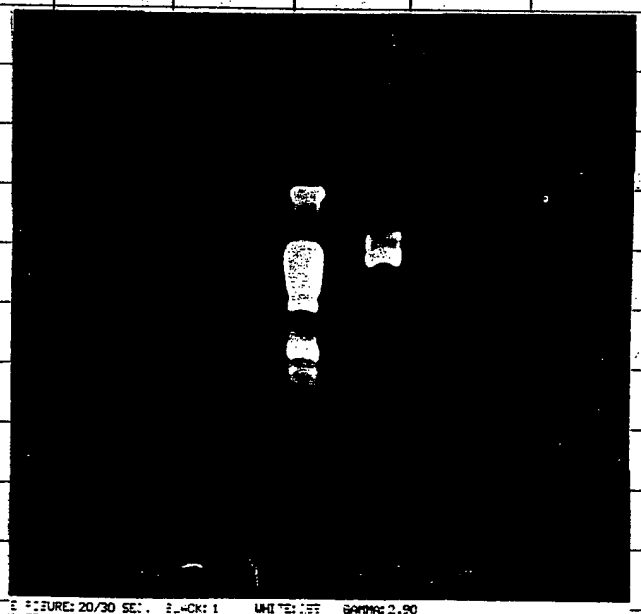
1) Digestion set up as follows: (2 rxns. made)

1 µl DNA (pFOX EGFP-N1)
1 µl EcoR1 Buffer
1 µl EcoR1
7 µl ddH_2O
10 µl tot. vol.

Digested for 1 hr. @ 37° C

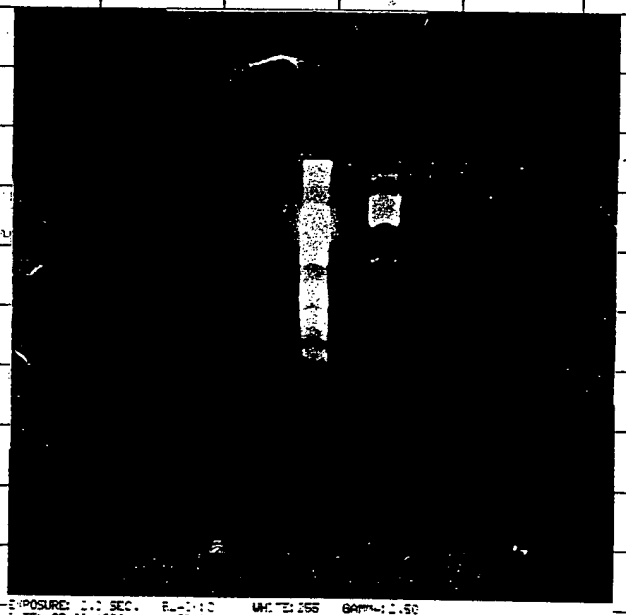
2) loaded onto 1.5% agarose gel:

* 20 µl marker
* 12 µl digestion spl



M 1 2

* where's the 850 bp band?
* funky gel!



M 1 2

* ~ 1000 bp band seen
* will redigest

↳ * put in EtBr bath 1µg/ml for 10'; wash for 5'

* lo weight band observed on

~~pFOXEGFP-N1~~ pS651-C1

Applied Biosystems
Model 373A
Version 1.2.0

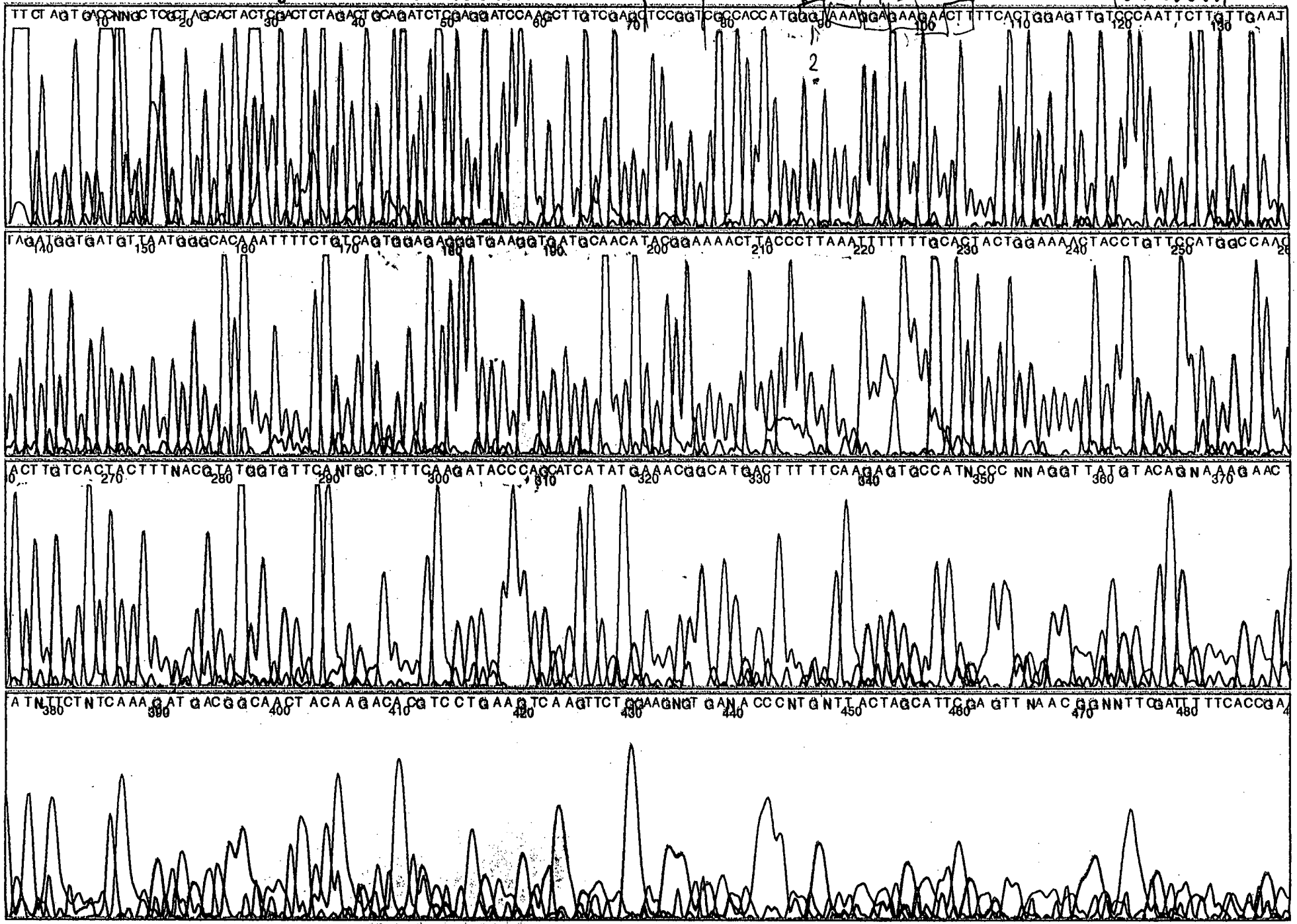
Sample 11
DyeTerminator{AnyPrimer}
Lane 11
Signal: G:179 A:126 T:106 C:30

Points 56[illegible] 6132 Base 1: 565
Instrume[illegible]907443
MS 2169

Wed, Jul 10, 1996 3:00 PM
X: 0 to 6887 Y: 0 to 1200
Spacing: 10.57

BspEI/AgeI

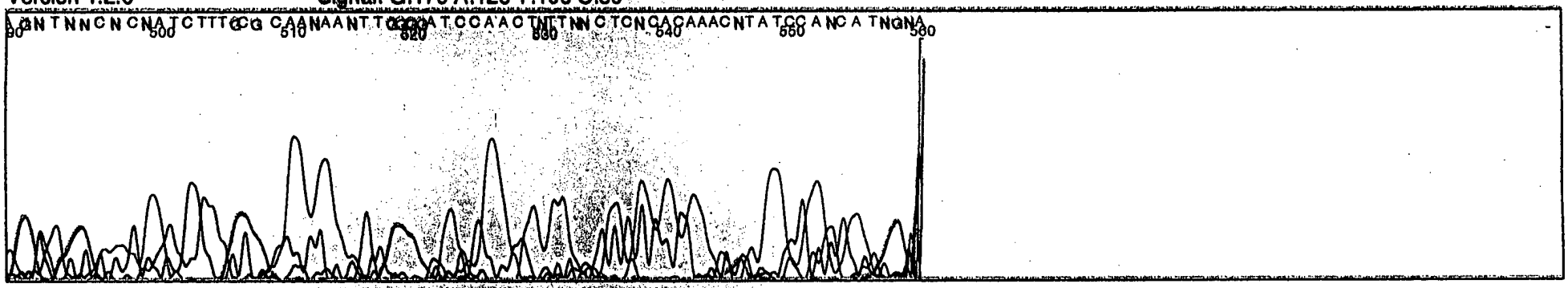
forward



Sample 11
DyeTerminator(AnyPrimer)
Lane 11
Signal: G:179 A:126 T:106 C:30

Points 56[illegible]o 6132 Base 1: 565
Instrume[illegible]907443
MS 2169

Wed, Jul 10, 1996 3:00 PM
X: 0 to 6887 Y: 0 to 1200
Spacing: 10.57



pBoxEGFP-N1

P3031-C1

Applied Biosystems
Model 373A
Version 1.2.0

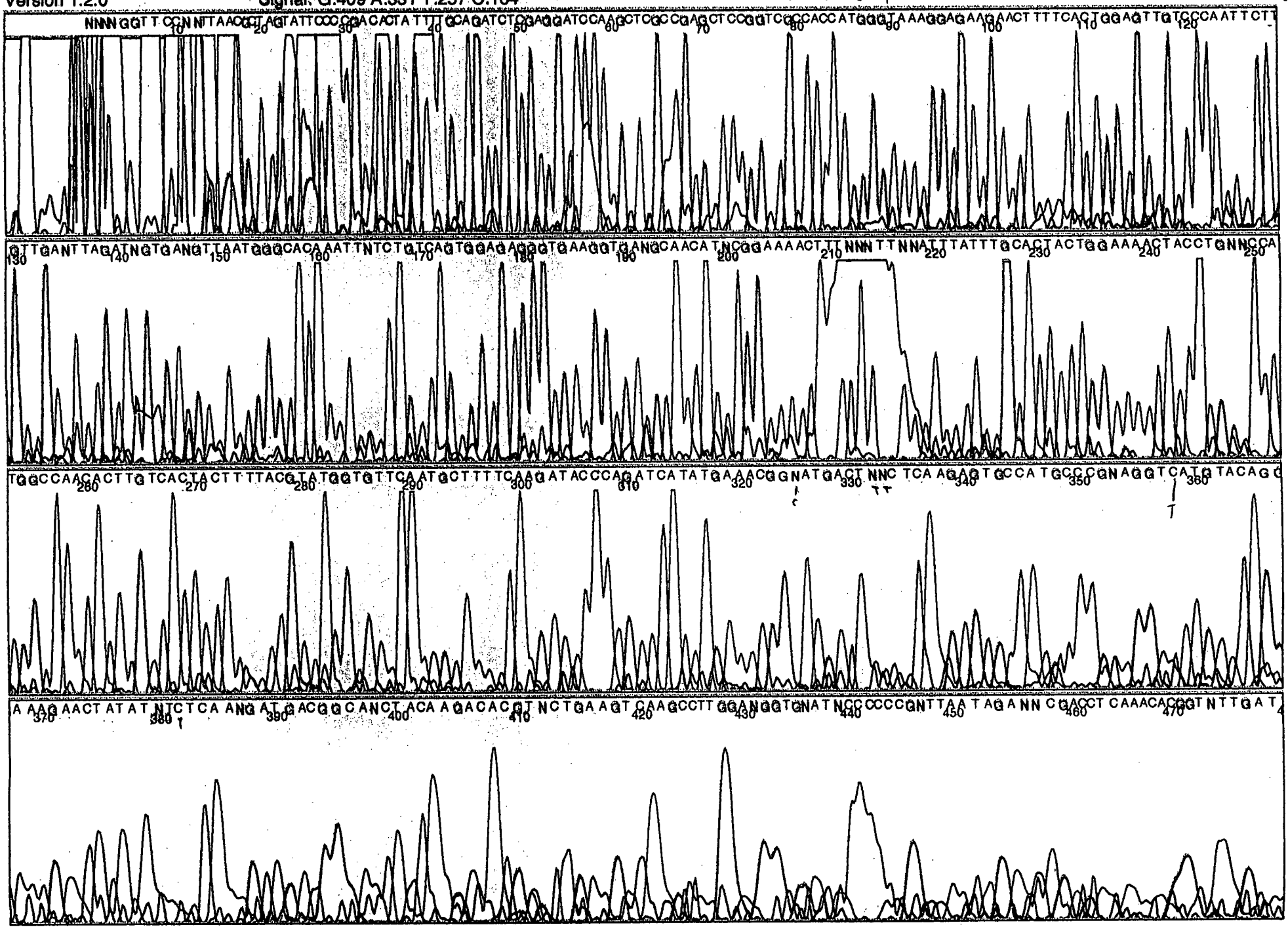
Sample 12
DyeTerminator{AnyPrimer}
Lane 12
Signal: G:409 A:331 T:257 C:164

Points 62[illegible] 6132 Base 1: 620
Instrumen[illegible]907443
MS 2170

Wed, Jul 10, 1996 3:00 PM
X: 0 to 6867 Y: 0 to 1200
Spacing: 10.48

Page [illegible] of 2

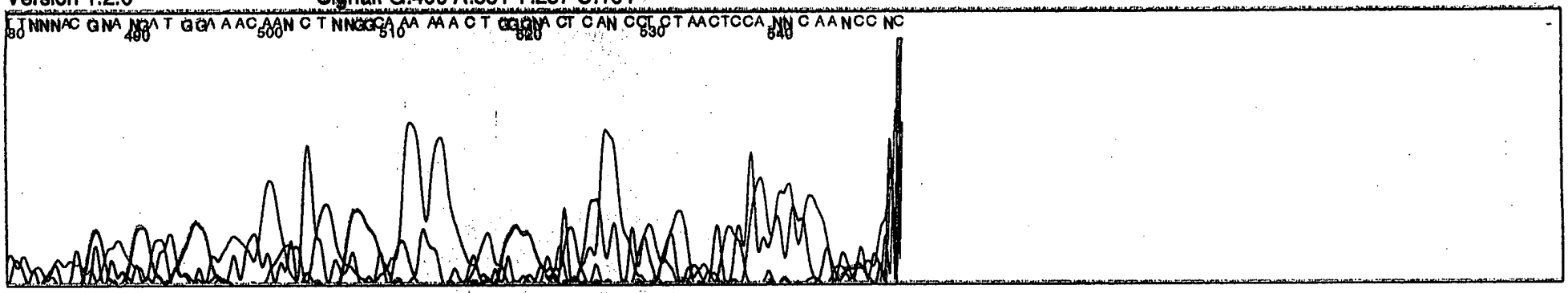
Rev



Sample 12
DyeTerminator{AnyPrimer}
Lane 12
Signal: G:409 A:331 T:257 C:164

Points 62[illegible]6132 Base 1: 620
Instrume[illegible]907443
MS 2170

Wed, Jul 10, 1996 3:00 PM
X: 0 to 6867 Y: 0 to 1200
Spacing: 10.48



Sac I Digest pFOXEGFP-N1

7-9-96

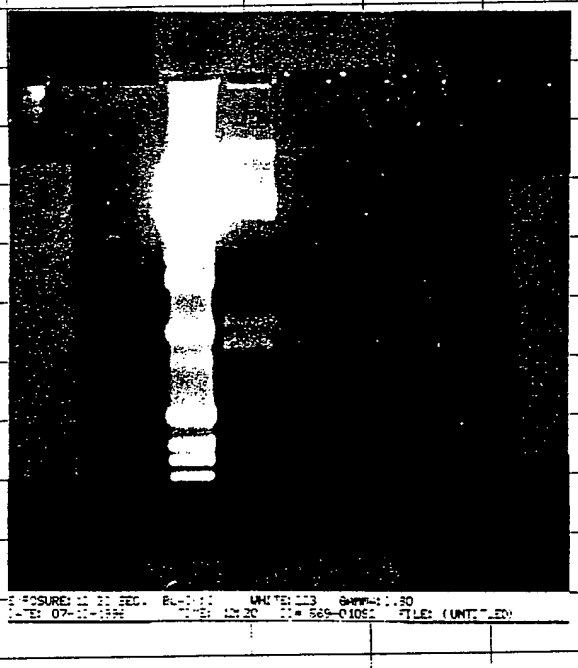
1) the following digest was setup:

10 µl DNA (pFOXEGFP-N1)
1 µl BSA (final conc. 2X)
4 µl Sac I
5 µl Buffer I
30 µl ddH_2O
50 µl total volume

* a second digestion w/ EcoRI was setup as previously described (1 µl EcoRI & 1 µl DNA); both digestions performed in PCR machine @ 37° for 4 hr.

7-10-96

EcoRI digest of pFOXEGFRN1 was run on 1.5% agarose gel:



* colony 24 #1

* 20 µl λ marker & 12 µl rxn. spl
* bad digestion; can see a lot of undigested plasmid; also observe the partial digest band ~ > 1000 bp
→ this may have resulted from incomplete digest to make 850 + 175 => 1025 bp band
* 850 bp (GFP & NLS) band observed

7-10-96

CIP of pFOXEGFP-N1

① CIP rxn. of Sac I digested vector was setup as follows: (added directly to digestion rxn.)

30 µl	10X CIP Buffer
219 µl	ddH_2O
1 µl	CIP
+ 50 µl	Sac I digest
300 µl	total vol.; incubate @ 37° for 30'

② add 1 µl additional CIP, then incubate @ 37° for 30'

③ stop rxn. by adding:

(39) 40 µl	ddH_2O
40 µl	10 X STE
20 µl	10% SDS
400 µl	total volume; heat @ 68° for 15'

④ Extract 2X w/ phenol/chloroform in 1:1 (v/v) ratio

* add phenol/chloroform; vortex; centrifuge @ hi speed for 5'

⑤ EtOH precipitate DNA w/ 1 ml cold EtOH; incubate in dry ice/EtOH bath for 15'; centrifuge 15'; wash w/ 1 ml 70% EtOH; resuspend in 50 µl 1X TE

Ligation of pFOXEGFP-N1 w/ human β-globin intron 7-10-96

1) ligation rxns. were setup as follows:

a) 1 µl plasmid (pFOXEGFP-N1)
2 µl insert (β-globin intron 175 bp)
1 µl ligase (400000 u/ml)
1 µl ligase buffer (10x)
5 µl ddH_2O
10 µl tot. vol.

b) 1 µl plasmid
6 µl insert
1 µl ligase
1.5 µl lig. buffer
5.5 µl ddH_2O
15 µl tot. vol.

c) control: 1 µl plasmid + 9 µl ddH_2O = 10 µl tot. vol.

* 175 bp β-globin human intron was not quantified

2) ligation rxns. were incubated @ 16° overnight

Transformation of HB101 cells w/ pFOXEGFP-N1 Ligation 7-11-96

1) cells thawed on ice and added directly to ligation rxns.; rest of transformation performed as previously described; plate 100 µl rxn. to each plate (LB + amp)

2) plate on LB+amp (100 µl) of
a) control b) 2 µl insert c) 6 µl insert
* incubate overnight (start 12:00 ⇒ 4pm
7-11-96 7-12-96)

Miniprep of pFOXSB

7-11-96

1) as described in Sheryl's protocol, except put on ice for 5' after adding NaOAc (3M pH 5.2)
2) after adding 100% EtOH, put in methanol/CO_2 bath for 15'; centrifuge @ hi speed for 3'
3) DNA resuspended in 30 µl 1x TE

NcoI Digestion of primary plasmids used to construct GFP plasmid

* sequence data of pFOXEGFP-N1 showed that the wrong plasmid may have been used to construct GFP plasmid; cutting w/ NcoI should determine whether the right plasmid (pEGFP-C1) and not the wrong, old plasmid (pS65T-C1) was used to construct GFP plasmid. The wrong plasmid pS65T-C1 will yield an extra low weight fragment (167 bp).

* sequence data found on next page

1) Digestion rxn. setup as follows:

Mix Made:
- 5 µl DNA
- 0.1 µl RNase
- 1 µl NcoI
- 1 µl Buffer 4
- 2.9 µl ddH_2O

10 µl tot. vol.

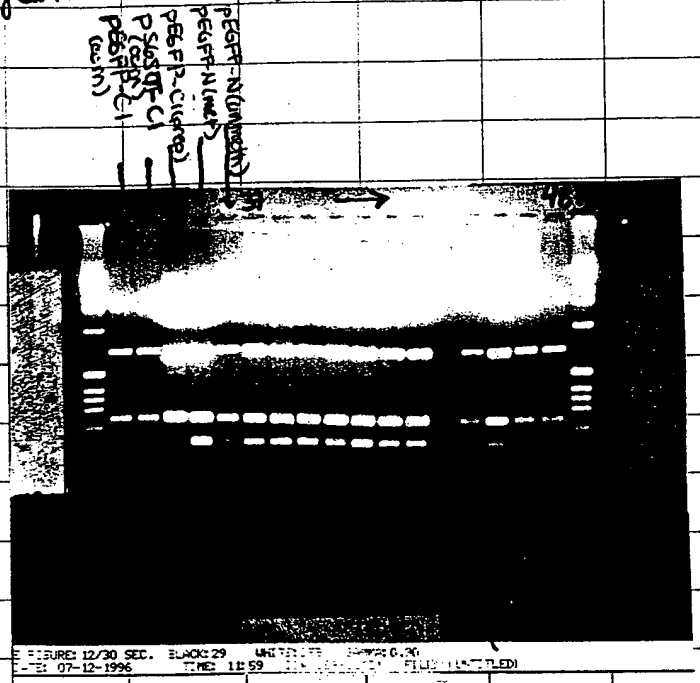
* for commercial plasmids pEGFP-C1 and pS65T-C1 only 1 µl DNA was used and added to 4 µl ddH_2O; this was added to 5 µl of mix w/ a tot. rxn. vol. of 10 µl

* also digested minis (pEGFP-N) # 37-48 (positives) @ 37° for 1 ½ hrs.; then stored in freezer

* digestion of pEGFP-C1 (prep); pEGFP-N (MIDI prep) and pEGFP-N (unmethylated) was performed

~~Not I~~ NcoI Digestion (cont'd) 7-12-96

i) digestion rxns. were run on 2% agarose gel : (120V)



lane order:

1) Marker 2) pEGFP-C1 (commercial) 3) pS65T-C1 (com)
4) pEGFP-C1 (prep) 5) pEGFP-N (methylated)
6) pEGFP-N (MIDI prep, unmethylated)

* used 20 µl marker
* ~ 12 µl spl loaded

→ presence of extra 167 bp fragment indicates minis made from old parent vector pS65T-C1
→ start over w/ pEGFP-C1 prep !

7-12-96

Annealing of Oligos

* MG528 & MG529 @ conc. of 1 ug/ul

1) added 1 ug (1 ul) of oligo MG528 and MG529 into rxn. w/ 2.5 ul Buffer H (Boehringer-Mannheim) to a total rxn. vol. of 25 ul
2) boiled in water bath for ~1 minute then kept in water bath as it ~~ego~~ temp. equilibrated to RT
   * no kinase
3) put @ 4°C for 2 hr. before using

Digestion of pEGFP-C1 (prep)

1) digestion rxn. setup as follows:

10 ul	pEGFP-C1 (prep)
2.5 ul	Bsp E1
2.5 ul	Bgl II
5 ul	Buffer 3
30 ul	ddH_2O
50 ul	total volume

2) incubate @ 37° for 4 hr.
3) phenol/chloroform and ethanol ppt. the DNA; resuspend in 30 ul 1XTE

Ligation of pEGFP-C1 (cut) w/ oligos MG528/MG529 — 7-12-96

* 3 dilutions of oligos were made:
  1:10 1:100 1:1000

1) * ligation rxn. set up as follows:
  1 µl cut plasmid (pEGFP-C1 cut w/ BspEI ; BglII)
  1 µl oligo dil'n (1:10 ; 1:100 ; 1:1000)
  1 µl T4 DNA ligase (400000 u/ml)
  1 µl ligase buffer (10x)
  6 µl ddH_2O
  10 µl tot. vol.
* control: 1 µl cut plasmid + 9 µl ddH_2O ⇒ 10 µl tot. vol.

2) Incubated @ 14-15° overnight

Transformation of DH1 cells w/ pEGFP-C1 ligation rxn. — 7-13-96

1) DH1 cells thawed on ice (~100 µl cells) and ligation rxns. added to cells; incubate in ice for 30', then heat shock cells
2) incubate @ 37° for 1 hr.; & plated on LB + kan plates which were incubated @ 37° overnight
  * 100 µl were plated on each plate

* very low transformation (1-2 colonies in 1:100 & 1:1000 dilns.; no colonies in 1:10 dil'n); will do a new ligation but use HB101 cells instead.

Ligation pEGFP-C1 (cut) w/ oligos

7-15-96

1) ligation rxn. setup as before and incubated @ RT for ~ 7 hr.
2) ligation rxn. were then transformed into HB101 cells; 100 µl ea. sample plated on Kan plates & incubated overnight @ 37°

7-16-96

3) started culture of cell colonies in 2 ml LB + kan and incubated 37° overnight
   * culture will be made from colonies w/ 1:10 dil'n of ligation rxn.

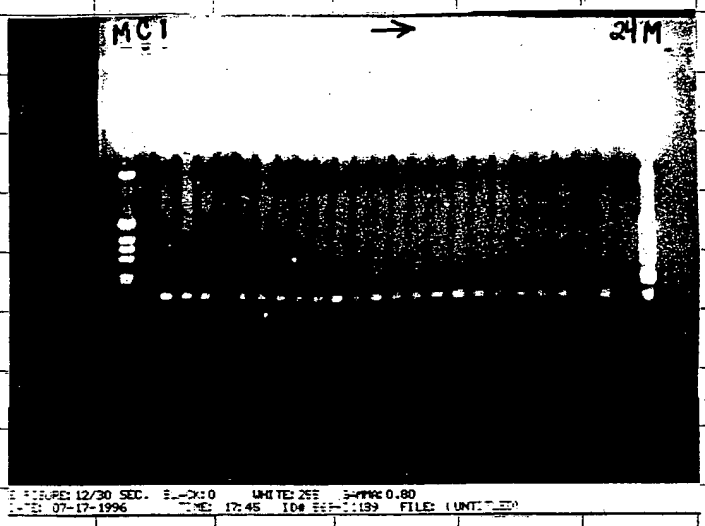
7-17-96

1) Miniprep of cultured cells containing ligation of pEGFP-C1 and MG528/MG529 oligos; DNA resuspended in 30 µl 1XTE
2) DNA digested w/ HinCII for 2½ hrs. @ 37° air incubator; digestion rxn. was setup as follows:

		30X
5 µl	DNA (pEGFP-C1)	
0.1 µl	RNase	3 µl RNase
0.5 µl	HinCII	15 µl HinCII
1 µl	Buffer 3	30 µl Buffer 3
.05 µl	BSA (mistake!) ⇐ should be 0.1 µl	1.5 µl BSA (100x)
3.4 µl	ddH$_2$O	100.5 µl ddH$_2$O
10 µl	tot. volume	150 µl tot. vol.

HinCII digestion of pEGFP-C1 minis 7-17-96

3) DNA run on 2% agarose gel @ 70←100 V:



* used 20 µl marker ; loaded 12 µl spl.

* see 147 bp band but no 267 bp or 302 bp bands since now working w/ pEGFP-C1 parent vector and not pS65T-C1 ; will react w/ other enzymes

Digestion of pEGFP-C1 (minis)

1) digestion rxns. were setup as follows:

5 µl	DNA	
0.1 µl	RNase A	3 µl
0.1 µl	BSA	3 µl
0.5 µl	NheI	15 µl
0.5 µl	XhoI	15 µl
1 µl	Buffer 2	30 µl
2.8 µl	dd H_2O	84 µl
10 µl	tot. vol.	150 µl

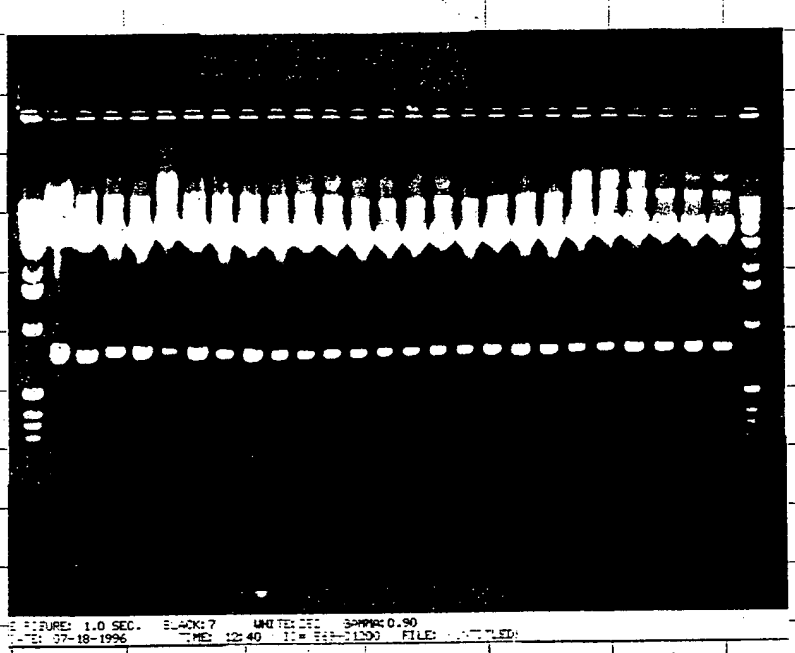
(30x)

2) incubated @ 37° in PCR for 4 hr.

(expected ⇒ ~~320~~ 322 bp-insert ; ~~650~~ 750 bp control)

XhoI & NheI digestion of pEGFP-C1 ligation 7-18-96

1) digestion rxn. run on 1.5% agarose gel @ 100 V:



* 20 µl marker ; 12.5 µl spl. loaded ; overloaded control (contains 5 µl pEGFP-C1 prep ; next time only use 1 µl diluted to 5 µl)
* col. 2 & 4 ⊕ appear slightly larger than control but cannot say definitively since resolution of 50 bp increase in correct construct is hard to resolve
* will passage 2 & 4 through DH1 and when these colonies are cut w/ AgeI & BspEI will also cut parent vector w/ same enzymes for a control ; again, correct fragment should be ~50 bp larger than control fragment

Transformation of DM1 w/ suspected pEGFP-C1 ligation 7-18-96

positives

1) transform DM1 cells w/ DNA from colonies 2 and 4; heat shock @ 42° C for 45 sec.; incubate in LB @ 37° for 1 hr. and plated on kan plates (100 µl cells)

pEGFP-C1 ligation & digestion 8-5-96

* Cultured colonies 2 & 4 in kan + LB 3ml
* miniprep of culture 4 and digestion w/ Age I

* pEGFP-C1 ligation rxn. was digested w/ Age I as follows:

	DNA	Buffer I	AgeI	RNase A	ddH_2O
1) pEGFP-C1 lig	40µl	10µl	4µl	1µl	45µl
2) pEGFP-C1 (cont)	1µl	1µl	1µl	—	7µl

* digested overnight @ RT

8-6-96

* EtOH ppt. AgeI digest'n rxn. & digested w/ BspEI for 4 hr. @ 37°
* miniprep done on colony 2 followed by Age I digestion as described above
  → 2 control rxns. setup as above

* for BspEI digestion, ligat'n rxn. digested as follows:

	DNA	Buffer ?	BspEI	RNase A	ddH2O
1) pEGFP-C1 lig	20µl	5µl	4µl	1µl	20µl
2) pEGFP-C1 (control)	10µl	5µl	1µl	—	34µl

@ 37° for 4 hr.

8-11-96

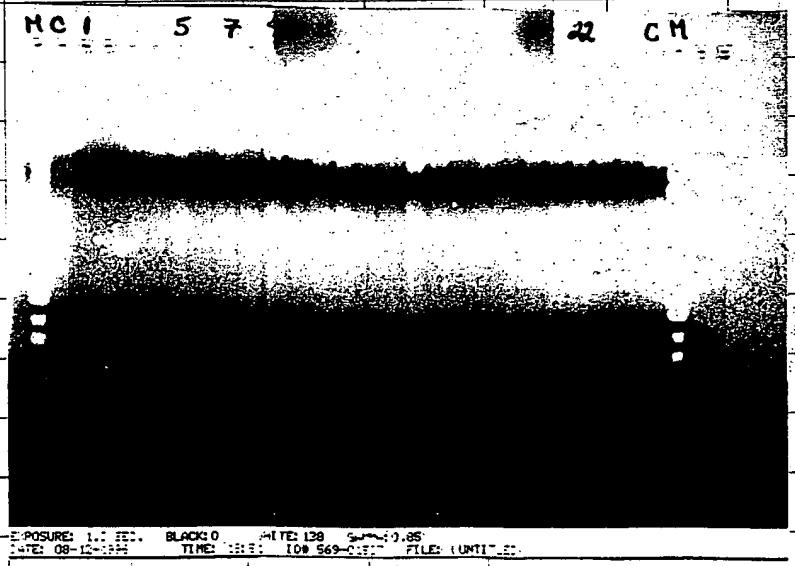
* started liquid culture of pEGFP-N1 in 3 ml LBamp

8-12-96

* Miniprep 1 ml pEGFP-N and digested as follows:

5 µl DNA (pEGFP-N)	3x	3 µl
0.1 µl RNase A	30X	3 µl
1 µl EcoRI		30 µl
1 µl EcoRI Buffer		30 µl
2.9 µl ddH_2O		87 µl
10 µl tot. vol.		

* incubate @ 37° for 4 hr.
* DNA used was resuspended in 30 µl 1X TE
* for control, used 1 µl pFCxSB DNA (2 controls made)
* for culture used to make DNA for this digestion, note that tubes 1, 2 & 17 were clear (i.e. apparently no bacteria) and so should be negatives on gel
* digestion run on 0.8% agarose gel:

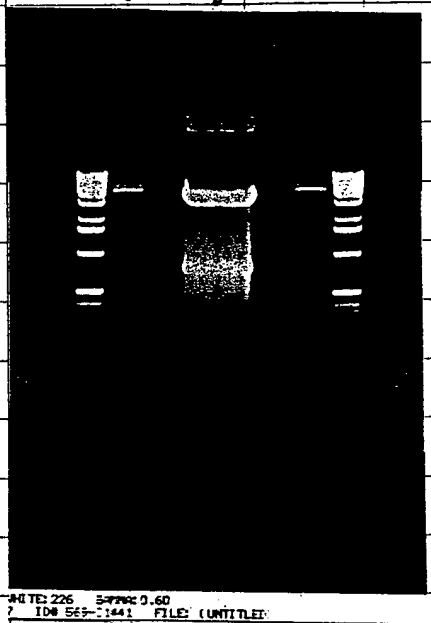


* 7 definite positives (i.e. has 850 & 175 band) #5, 7, 9-11, 20 & 22; possible positives include #1 & 13
⇒ will be pEGFP-N1

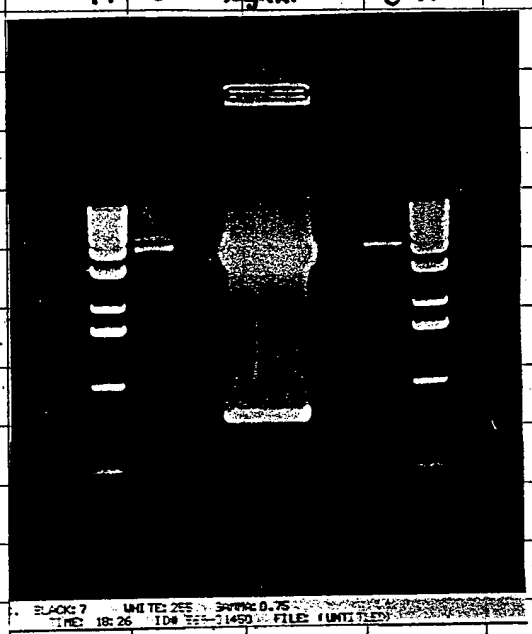
Digestion of Colony 2 w/ BspE1 8-7-96

* digestion rxn. analyzed on 1.5% agarose gel (100V):

M C lig.rxn. C M



M C lig.rxn. C M



→ pEGFP-N1

* negative ☹

* next time load less of ligation; can always run ~~a second~~ in 2 lanes ☺

* same gel allowed to run for longer time to improve resolution
* though control has very weak signal they can still be discerned to be smaller than lig'n rxn.
* ☺ band isolated; purified w/ Qiagen column; resuspend in 50 ul TE

Ligation of pFOXSB & pEGFP-N 750 fragment 8-8-96

Label	Contents				
	ddH$_2$O	BspEI cut & CIP pFOXSB	pEGFP-N frag 750 bp	ligase	lig buffer
	2 µl	5 µl	2 µl	1 µl	1 µl
	5 µl	2 µl	5 µl	↓	↓
	5 µl	2 µl	↓		
	10 µl	1.5 ~~µl~~	10 µl	↓	1.5 µl
(control)	C	9 µl	—	—	—

* rxn. run @ RT for 5 hr.
* transformed into HB101 cells & plated on amp+
* ~~miniprep~~ EtOH ppt & digestion w/ Bsp EI of colony 4 (from yesterday); will analyze if positive (has 750 band) on 1.5% agarose gel:

* DNA lost during ppt.

8-11-96

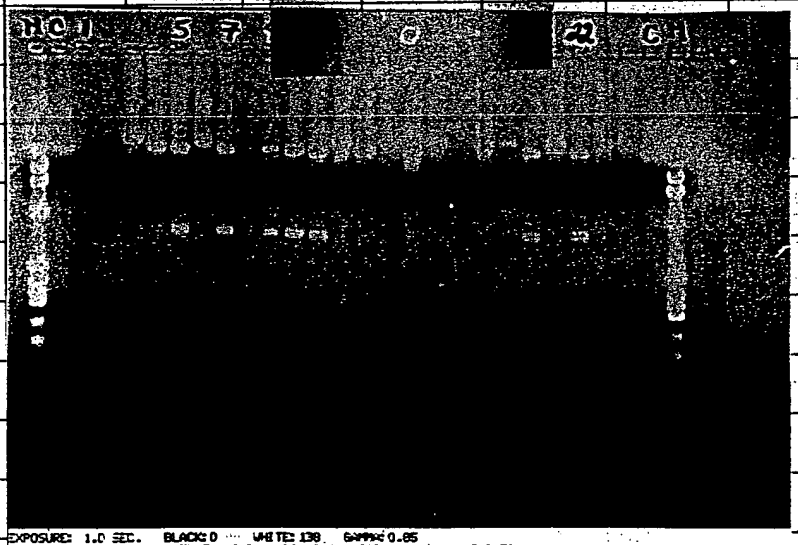
* started liquid culture of pE6FP-N1 in 3 ml LBtamp

8-12-96

* Miniprep 1 ml pE6FP-N and digested as follows:

5 µl DNA (pE6FP-N)	30X	3 µl
0.1 µl RNase A		3 µl
1 µl EcoRI		30 µl
1 µl EcoRI Buffer		30 µl
2.9 µl ddH_2O		87 µl
10 µl tot. vol.		

* incubate @ 37° for 4 hr.
* DNA used was resuspended in 30 µl 1X TE
* for control, used 1 µl pFOxSB DNA (2 controls made)
* for culture used to make DNA for this digestion, note that tubes 1, 2 & 17 were clear (i.e. apparently, no bacteria) and so should be negatives on gel
* digestion run on ~~0.8%~~ 1.5% agarose gel:



* 7 definite positives (i.e. has 850 & 175 band) #5, 7, 9-11, 20 & 22; possible positives include #4 & 13
→ will be pE6FP-N1

8-13-96

* pEGFP-N1 from miniprep digested w/ SacI:

27 µl	DNA (#10 pEGFP-N1)
1 µl	BSA
1 µl	RNase A
4 µl	SacI
5 µl	Buffer I
12 µl	ddH_2O
50 µl	

* digested @ 37° for 4 hr.
* reculture #5 & #10 into ~~new~~ fresh LB+amp (3 ml)

* pEGFPN1-SacI digest CIP 2x @ 37° for 30' w/ 1 µl CIP

in following rxn:	
50 µl	SacI digest
30 µl	10x CIP buffer
1 µl	CIP
219 µl	ddH_2O
300 µl	tot. vol.

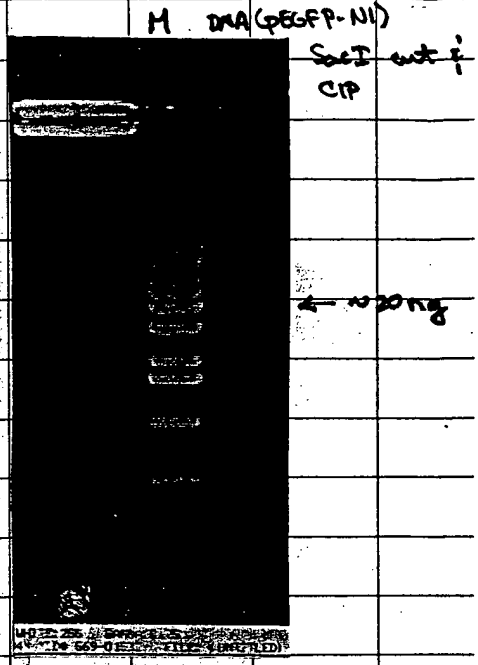
* CIP rxn. Stop by adding:

and heating @ 68° for 15'	
39 µl	ddH_2O
40 µl	10X STE
20 µl	10% SDS
400 µl	tot. vol. w/ SacI digest

* extract w/ phenol/chloroform 2x; EtOH ppt & resuspend in 15 µl TE (1x)
* run on 1% agarose gel ~~& purified for ligation rxn:~~ to assay concentration (1 µl DNA assayed) w/ 6 µl marker (1x)

8-13-96

* Assay for concentration of pEGFP-N1 cut SacI & CIP on 1% agarose gel ; 6 µl marker (1x) :



* ~20 ng/µl pEGFP-N1 to be used for ligation

* Ligation reactions setup as follows:

Name	pEGFP-N1 cut SacI/CIP	insert (intron)	Ligase Buffer (10x)	Ligase 400000u	ddH2O
2 µl	3 µl	2 µl	1 µl	1 µl	3 µl
6 µl		6 µl	1.5 µl	↓	3.5 µl
control	↓	—	—	—	7 µl

* ligated @ 15° overnight

8-14-96 * transformed into 100 µl HB101 cells & plated 100 µl onto LB+amp @ 37° overnight

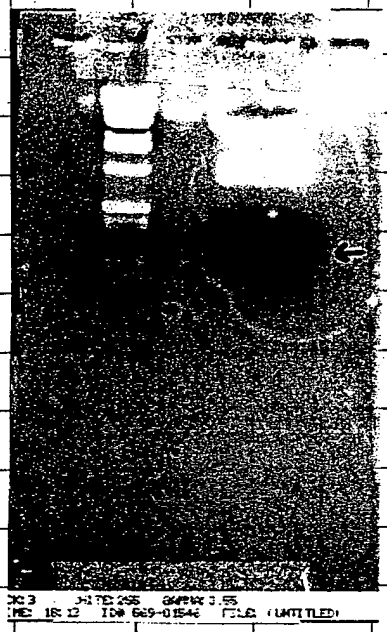
* Qiagen column (20-tip) on colonies 5 & 10 (pEGFP-N1) and resuspended in 20 µl 1x TE

* Digested pFOXCAT2 w/ SacI to isolate 175 bp β-globin intron :

50 µl PFC2
4 µl SacI
1 µl BSA
7.5 µl Buffer I
12.5 µl ddH_2O
75 µl tot. vol.
@ 37° for 4 hr.

8-14-96

* pFC2 SacI digest to isolate 175 bp intron run on 1.5% gel:



* isolated & purified 175 bp β-globin intron w/ Qiagen; resuspended in 50 µl 1x TE

8-15-96

* plates of pEGFP-N1 ligation w/ 175 bp intron show hi transformation efficiency; will culture 12 colonies each (w/ total of 24 cultures) from 2 µl & 6 µl plates into 3 ml LB+ amp; 37° overnight
* cut #5 & #10 pEGFP-N1 w/ SacI and kept in freezer; digest 10 µl ea. DNA w/ 4 µl enzyme in 50 µl rxn.

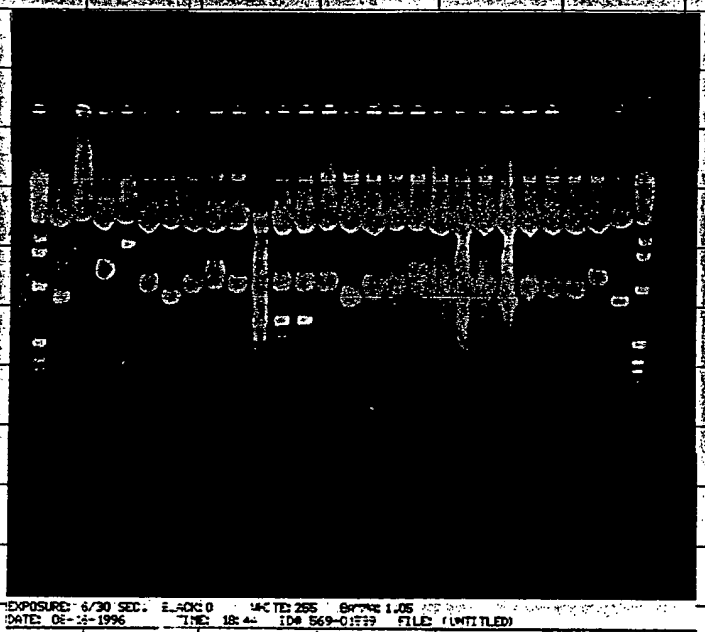
8-16-96

* minis of suspected pEGFP-N2 ; minis on 1 ml culture; after addt'n of 900 µl 100% EtOH put in EtOH bath for 15' and centrifuged for 10' w/ subsequent aspiration ; resuspended in 30 µl 1X TE

* susp. pEGFP-N2 digest w/ EcoRI

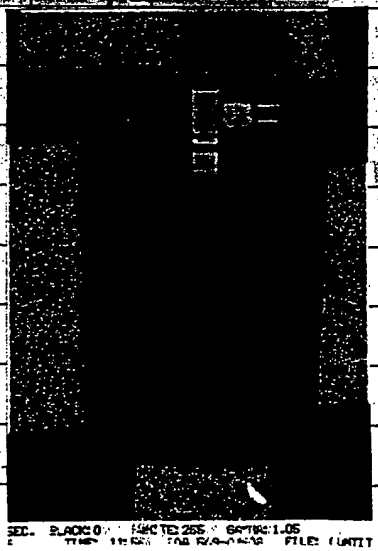
5 µl DNA (susp. pEGFP-N2 ;		pEGFP-N1 control used 1 µl)
0.1 µl RNase A	} 35x	3.5 µl RNase A
1 µl EcoRI		35 µl EcoRI
1 µl EcoRI Buffer		35 µl EcoRI Buffer
2.9 µl ddH_2O		101.5 µl ddH_2O
10 µl tot. vol.		175 µl

* digest run on 1.5% gel :



* recultured #4, 6, 19, 22, 23 in 3ml LBamp

* Qiagen column on #4 & 6



#4 ~180 ng
#6 ~60 ng

8-19-96

* pEGFP-N2 digested again w/ EcoRI & set up dbl digestion w/ EcoRI & BspM1 (will reppt.)

5 µl DNA
1 µl EcoRI (BspM1)
1 µl EcoRI Buffer (Buffer 2)
3 µl ddH_2O
10 µl tot. vol.

* digested pFC2-482 rglcZ w/ HindIII & BglII

30 µl DNA
5 µl Buffer B (Boehringer Buffer)
2.5 µl HindIII
2.5 µl BglII
10 µl ddH_2O
50 µl tot. vol.

dbl @ 37° 4 hr.

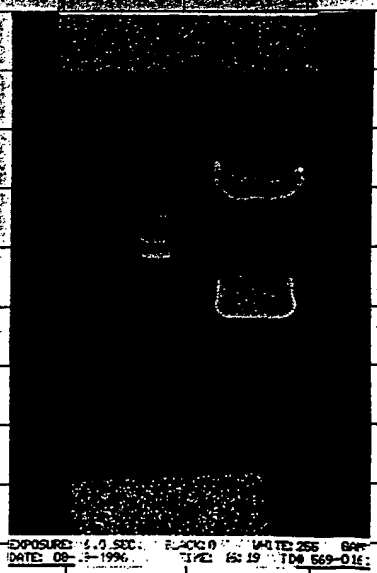
* second set dbl digestion on #4, 6, 8, 12, 14, 15, 21-23

15 µl DNA
1 µl RNase A
2 µl BspMI
10 µl Buffer 2
72 µl ddH_2O
100 µl tot. vol.

10x

incubate 37° overnight

* digested pFC2-482 rglcZ run on 1% agarose and purified ~730 bp fragment:
* resuspended in 50 µl TE



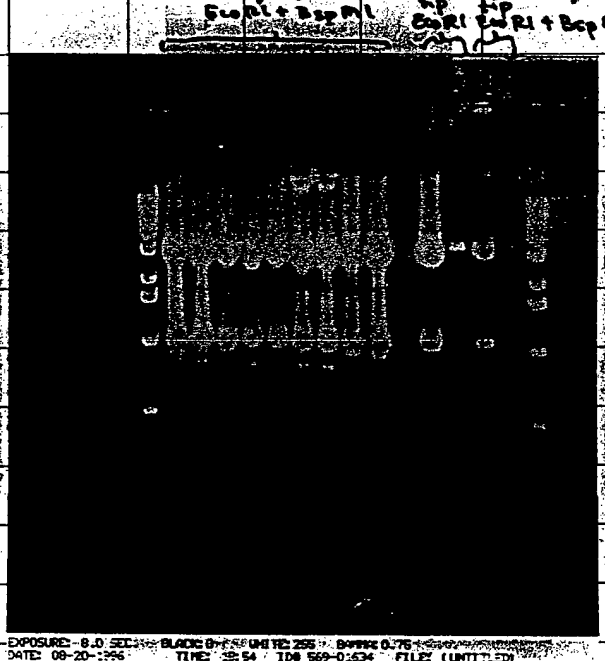
8-20-96

Digestion w/ Bsp MI & EcoRI to check for intron orientation:

* yesterday's Bsp MI rxn. phenol / chloroform extracted (1X) & ppt. w/ cold EtOH; resuspended in 10 µl 1XTE; digested w/ EcoRI as follows:

10 µl DNA		
1 µl EcoRI	15×	15 µl EcoRI
2 µl Buffer		20 µl Buffer
0.1 µl RNase A		1.5 µl RNase A
6.9 µl ddH_2O		103.5 µl ddH_2O
20 µl tot. vol		

* digested 4 hrs 37°
* run on 1.5% agarose gel; culture #4 & 23

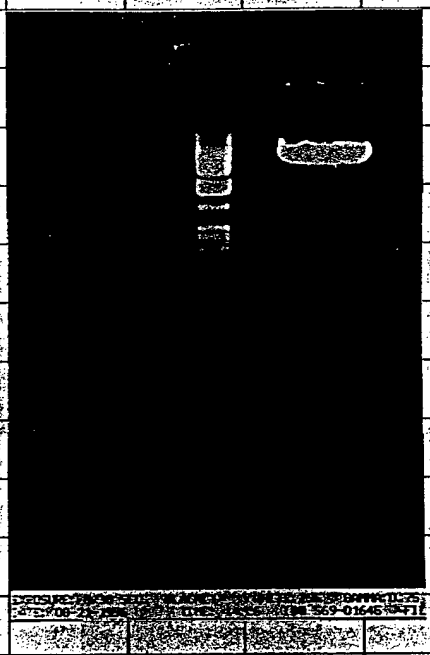


* can see 905 bp band on #4 (tip) and can be discerned for #8, 22, 23; bad digestion w/ Bsp MI since can't see 250 bp band for negatives # 1.2, 14, 15, 21

8-21-96

* digested pEGFP-N2 w/ HindIII & BglII so can ligate pFC2-482 rglc Z:

10 µl DNA (~10 µg)
2.5 µl HindIII
2.5 µl BglII
5 µl Buffer B
30 µl ddH_2O
50 µl tot. vol. @ 37° for 4 hr.



* run on 0.8% agarose gel; Qiagen gel extract vector & resuspend in 50 µl
* setup ligations w/ -482 rglc Z 73C fragment

cut EGFP	insert	ligase	lig buffer	ddH_2O
1 µl	2 µl	1 µl	1 µl	5 µl
↓	5 µl	↓	1.5 µl	6.5 µl

* control = 1 µl cut EGFP + 9 µl ddH_2O
* ligations incubate @ 15° overnight

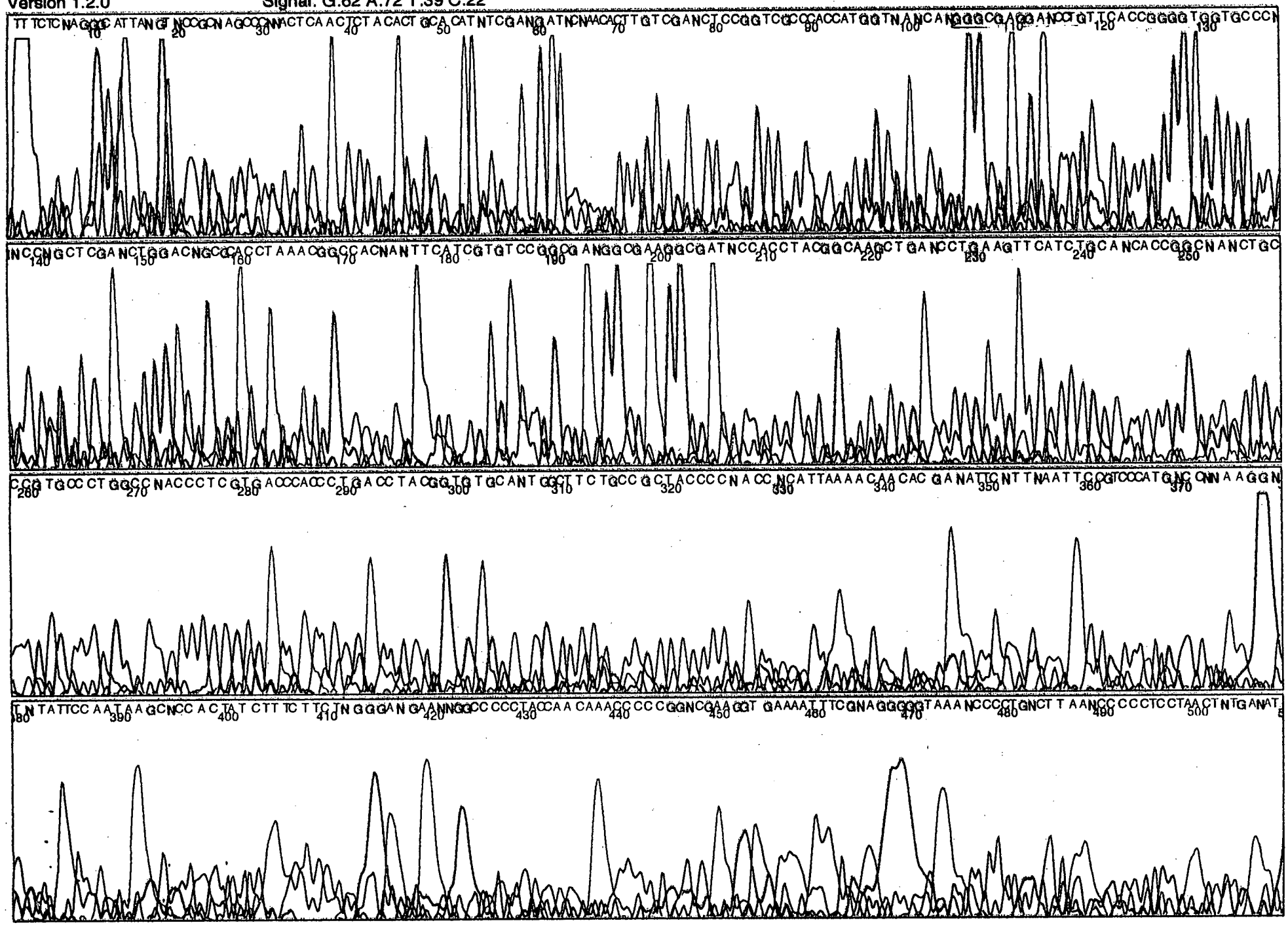
8-22-96

* plated ligt'n rxns onto amp+LB plates (100 µl ea.)

8-23-96

* started 24 cultures in ~3 ml LB+amp
* picked from "2 µl insert" plate
* did not observe hi transformation efficiency

10/1. mmis on CMV. 4U RIP. Kg/C. w GFP. V W RI (Janet).

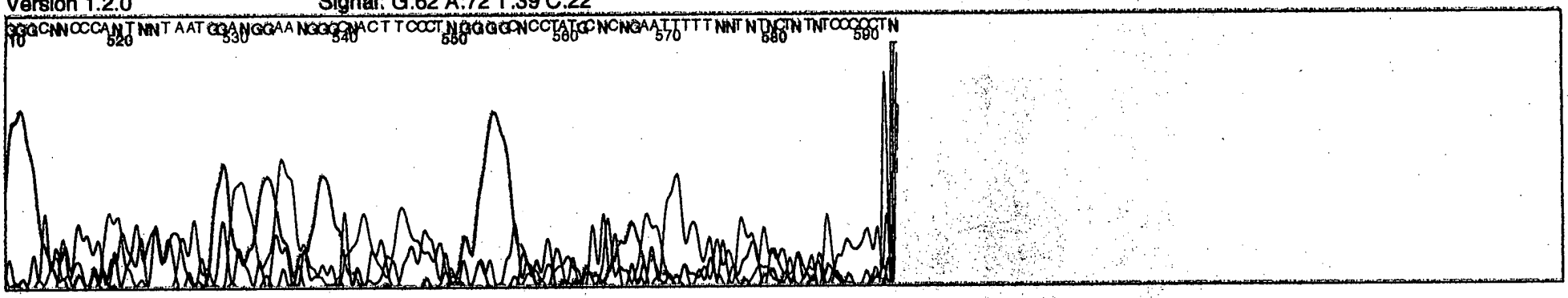


Applied Biosystems
Model 373A
Version 1.2.0

Sample 07.1
DyeTerminator{AnyPrimer}
Lane 7
Signal: G:62 A:72 T:39 C:22

Points 60[illegible] 6184 Base 1: 604
Instrument #907443
FD 2315

Wed, Aug 21, 1996 2:59 PM Page [illegible] of 2
X: 0 to 6858 Y: 0 to 1200
Spacing: 10.65

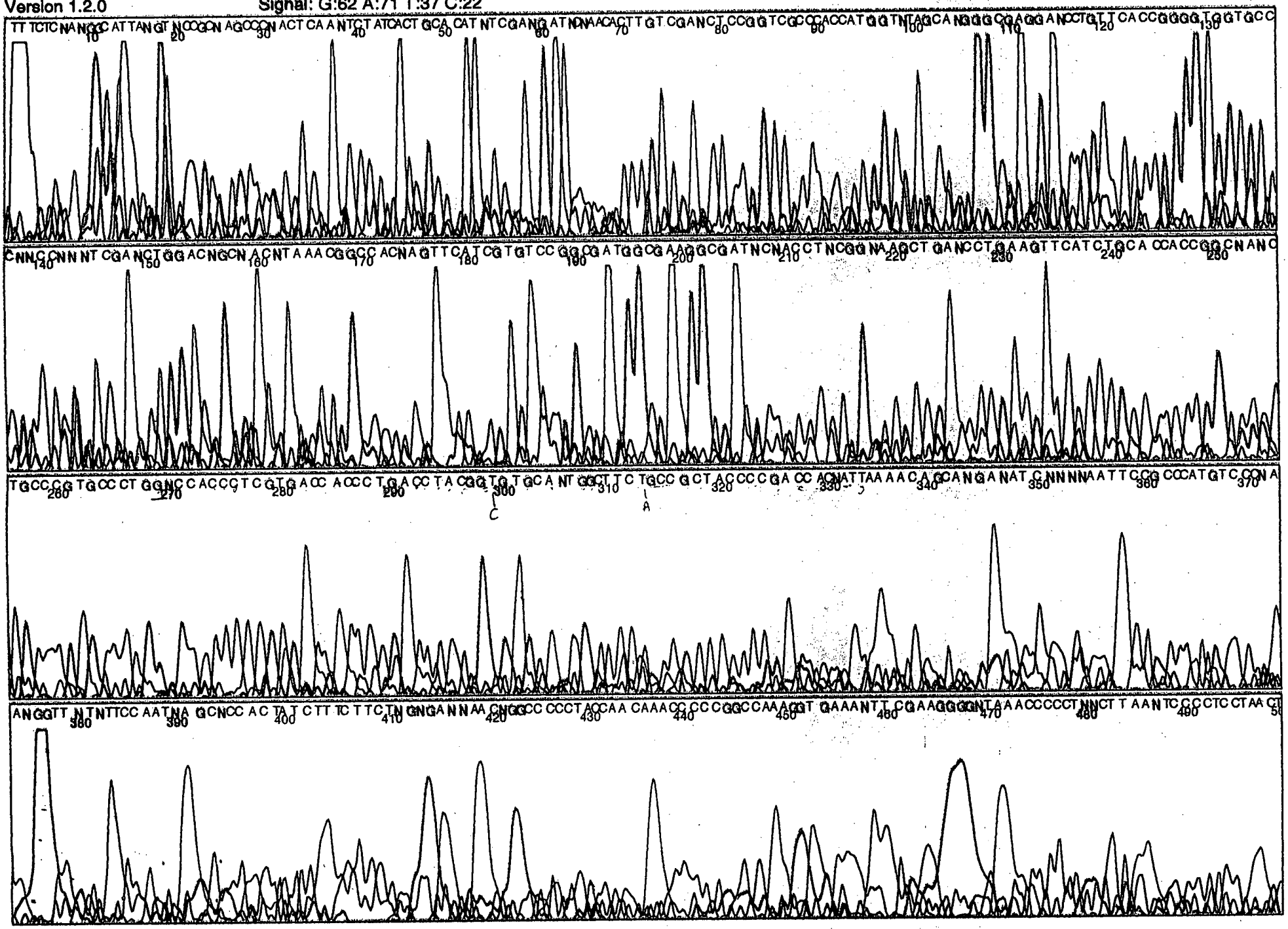


Applied Biosystems
Model 373A
Version 1.2.0

Sample 08
DyeTerminator{AnyPrimer}
Lane 7
Signal: G:62 A:71 T:37 C:22

Points 60 6184 Base 1: 604
Instrument #907443
FD 2316 5

Wed, Aug 21, 1996 2:59 PM Page of 2
X: 0 to 6947 Y: 0 to 1200
Spacing: 10.51

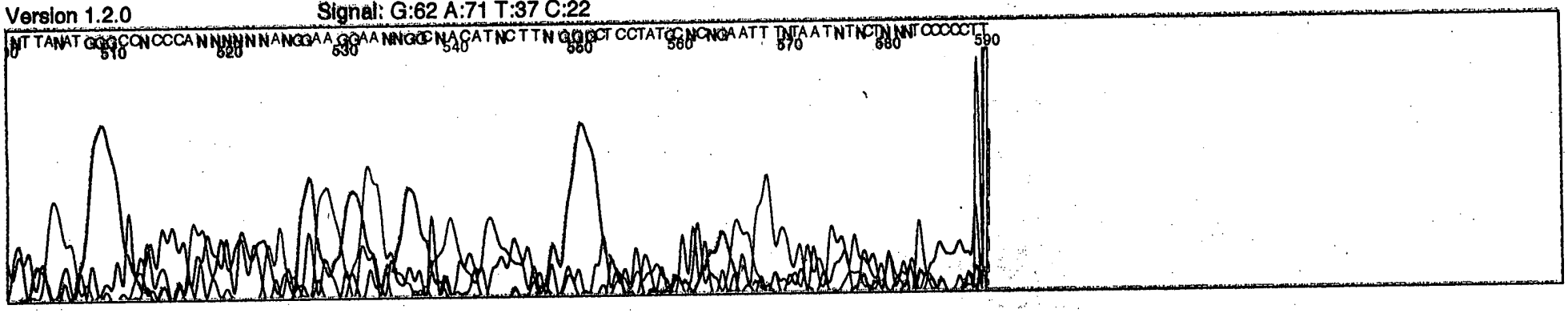


Applied Biosystems
Model 373A
Version 1.2.0

Sample 08
DyeTerminator{AnyPrimer}
Lane 8
Signal: G:62 A:71 T:37 C:22

Points 60[illegible] 6184 Base 1: 604
Instrument #907443
FD 2316

Wed, Aug 21, 1996 2:59 PM Page [illegible] of 2
X: 0 to 6947 Y: 0 to 1200
Spacing: 10.51



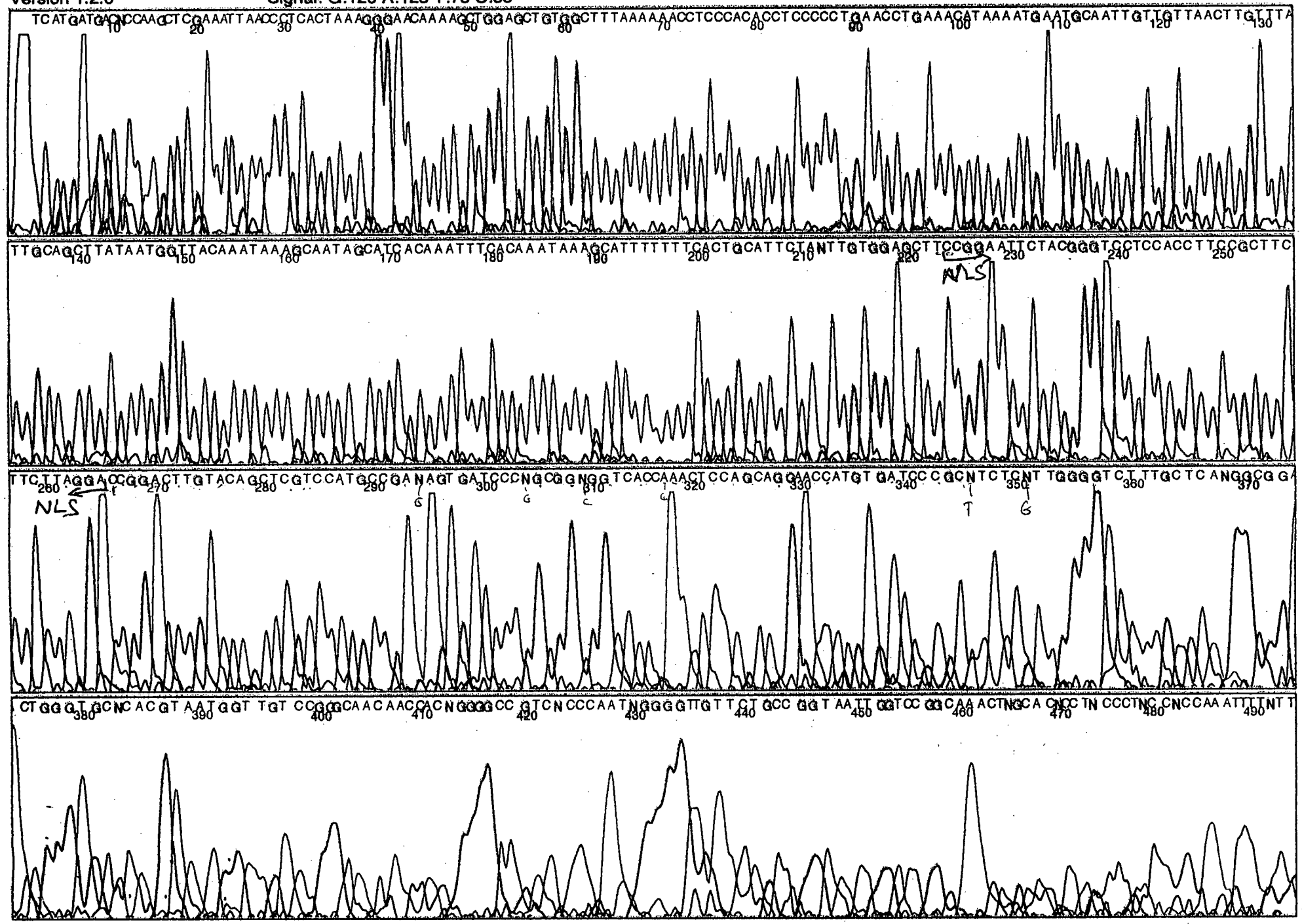
pEGFP-N1 reverse

Applied Biosystems
Model 373A
Version 1.2.0

Sample 08.1
DyeTerminator{AnyPrimer}
Lane 8
Signal: G:120 A:125 T:73 C:35

Points 69[illegible] 6184 Base 1: 692
Instrumen[illegible]907443
FD 2316

Wed, Aug 21, 1996 2:59 PM Page [illegible]f 2
X: 0 to 6561 Y: 0 to 1200
Spacing: 10.93

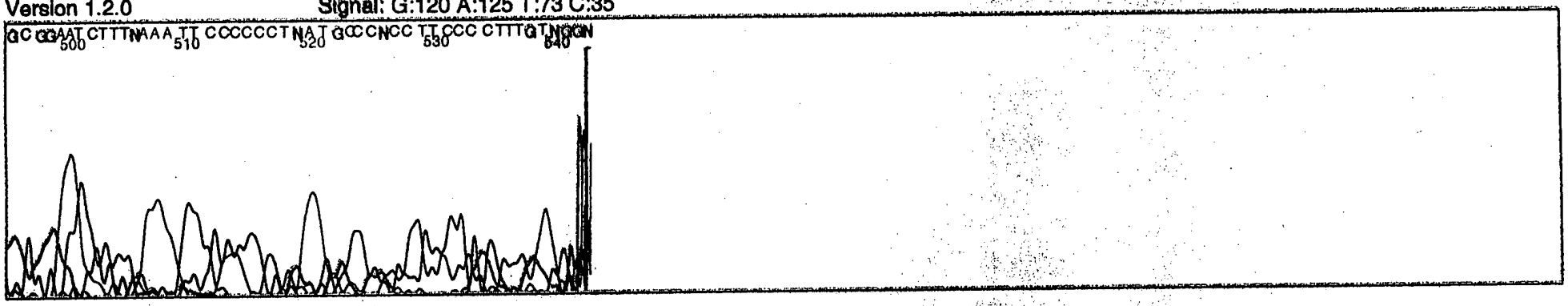


Applied Biosystems
Model 373A
Version 1.2.0

Sample 08.1
DyeTerminator{AnyPrimer}
Lane 8
Signal: G:120 A:125 T:73 C:35

Points 69[illegible] 6184 Base 1: 692
Instrumen[illegible]907443
FD 2316

Wed, Aug 21, 1996 2:59 PM
X: 0 to 6561 Y: 0 to 1200
Spacing: 10.93

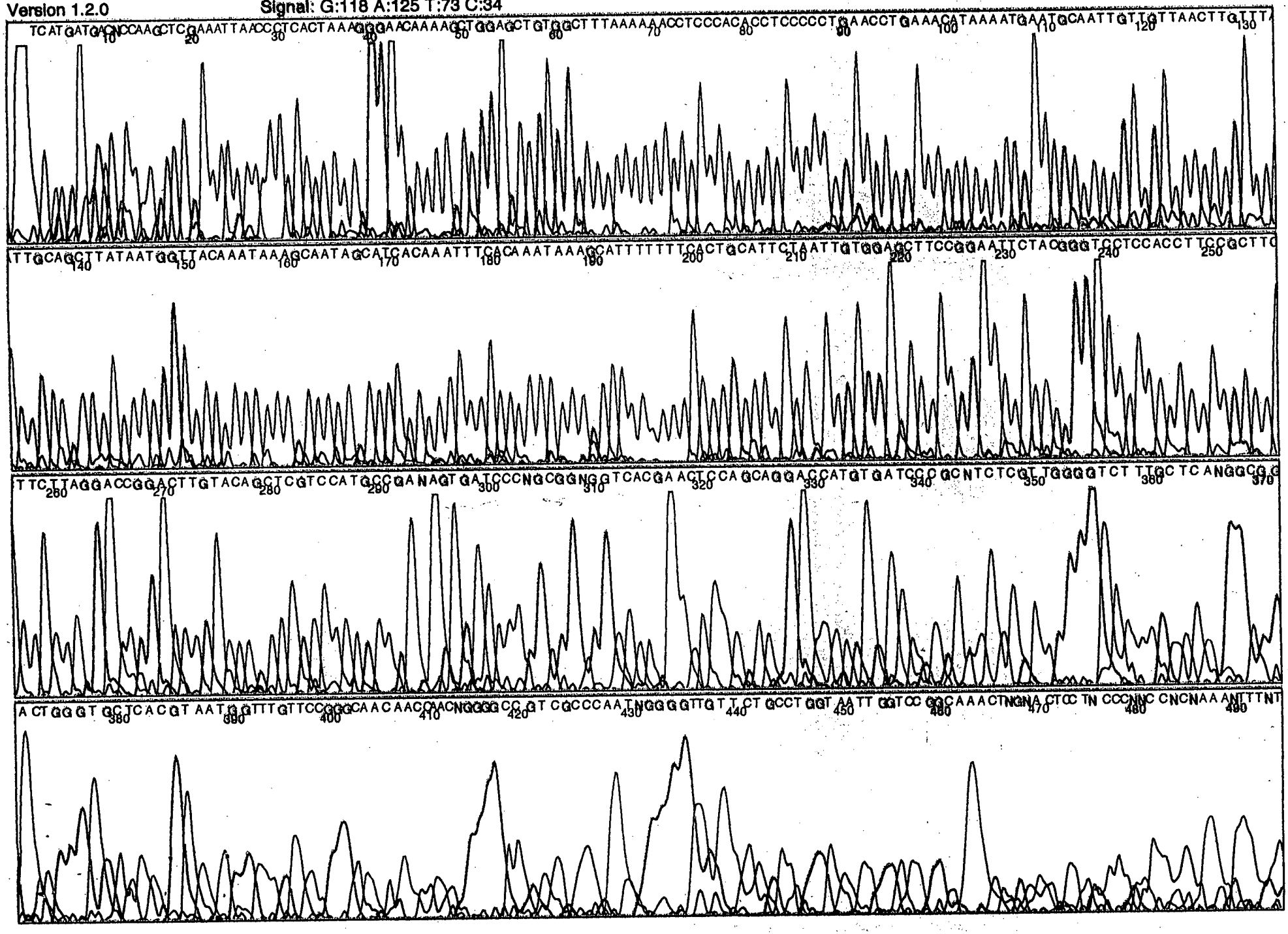


Applied Biosystems
Model 373A
Version 1.2.0

Sample 09
DyeTerminator{AnyPrimer}
Lane 08
Signal: G:118 A:125 T:73 C:34

Points 68 6184 Base 1: 692
Instrument #907443
~~RC 2317~~ FD 2316

Wed, Aug 21, 1996 2:59 PM Page 1 of 2
X: 0 to 6575 Y: 0 to 1200
Spacing: 10.91

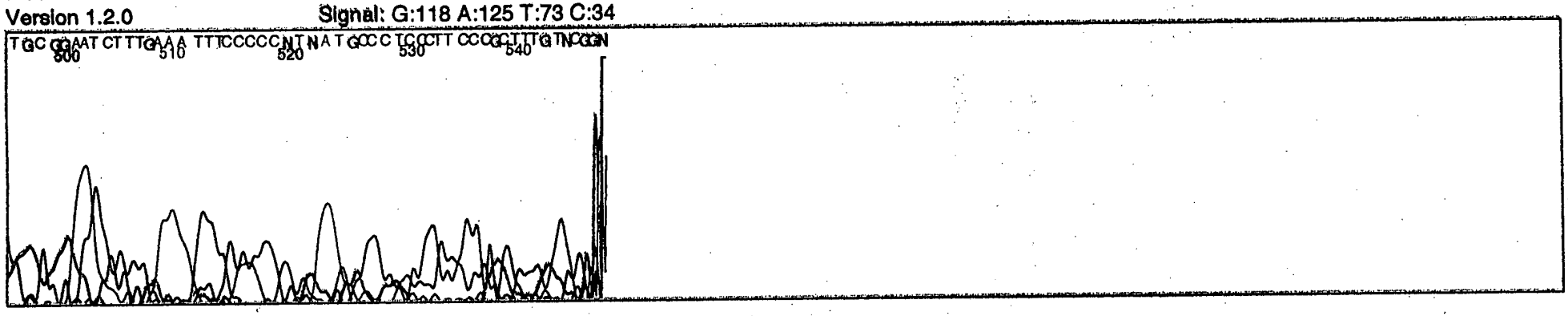


Apple Biosystems
Model 373A
Version 1.2.0

Sample 09
DyeTerminator{AnyPrimer}
Lane 9
Signal: G:118 A:125 T:73 C:34

Points 6[illegible] 6184 Base 1: 692
Instrument #907443
RC 2317

Wed, Aug 21, 1996 2:59 PM
X: 0 to 6575 Y: 0 to 1200
Spacing: 10.91



pCDFF-Na forward

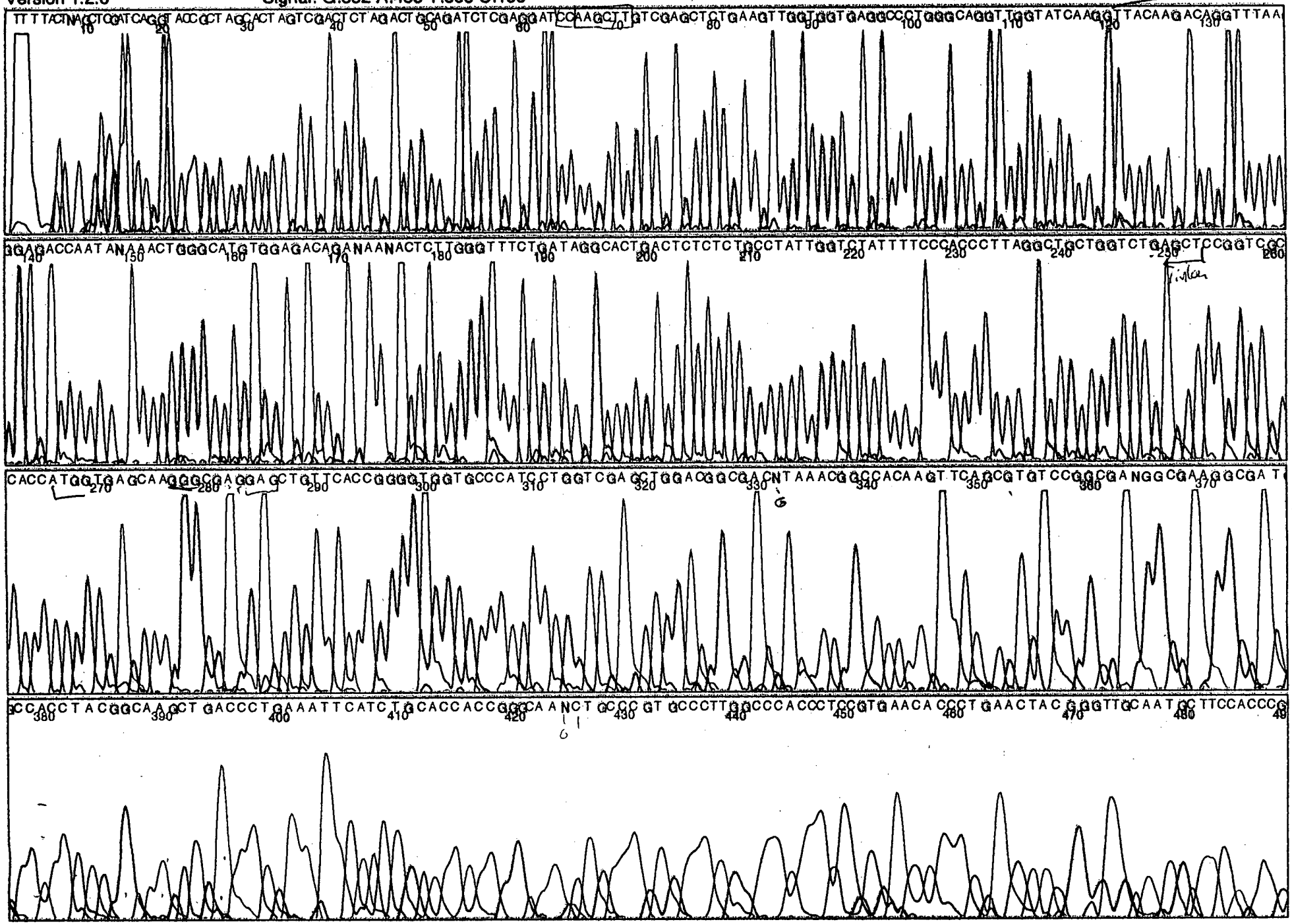
Applied Biosystems
Model 373A
Version 1.2.0

Sample 19
DyeTerminator{AnyPrimer}
Lane 19
Signal: G:352 A:485 T:305 C:158

Points 69[illegible]6184 Base 1: 691
instrumen[illegible]907443
~~Control pGEM~~ FD 2326

Wed, Aug 21, 1996 2:59 PM Page [illegible]of 2
X: 0 to 6503 Y: 0 to 1200
Spacing: 11.03

OK

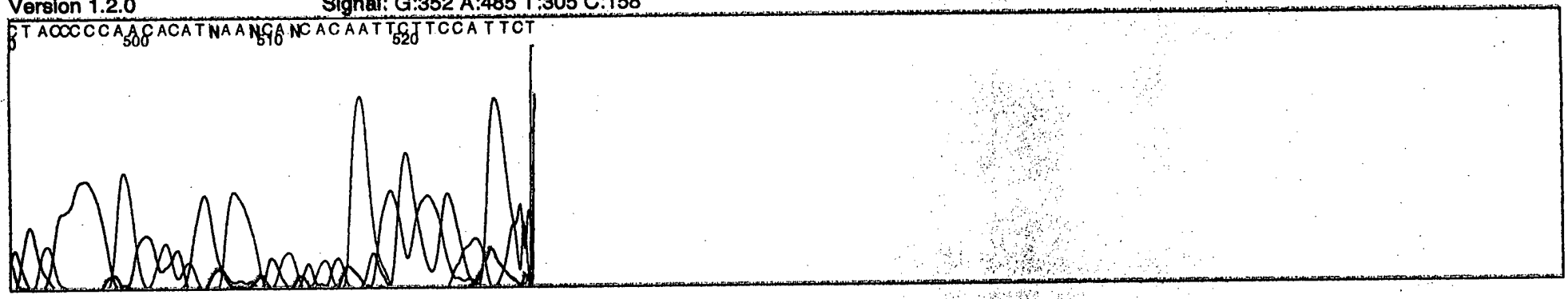


Applied Biosystems
Model 373A
Version 1.2.0

Sample 19
DyeTerminator{AnyPrimer}
Lane 19
Signal: G:352 A:485 T:305 C:158

Points 69[illegible] 6184 Base 1: 691
Instrument #907443
Control pGEM

Wed, Aug 21, 1996 2:59 PM
X: 0 to 6503 Y: 0 to 1200
Spacing: 11.03



pFOXE6FP-W2

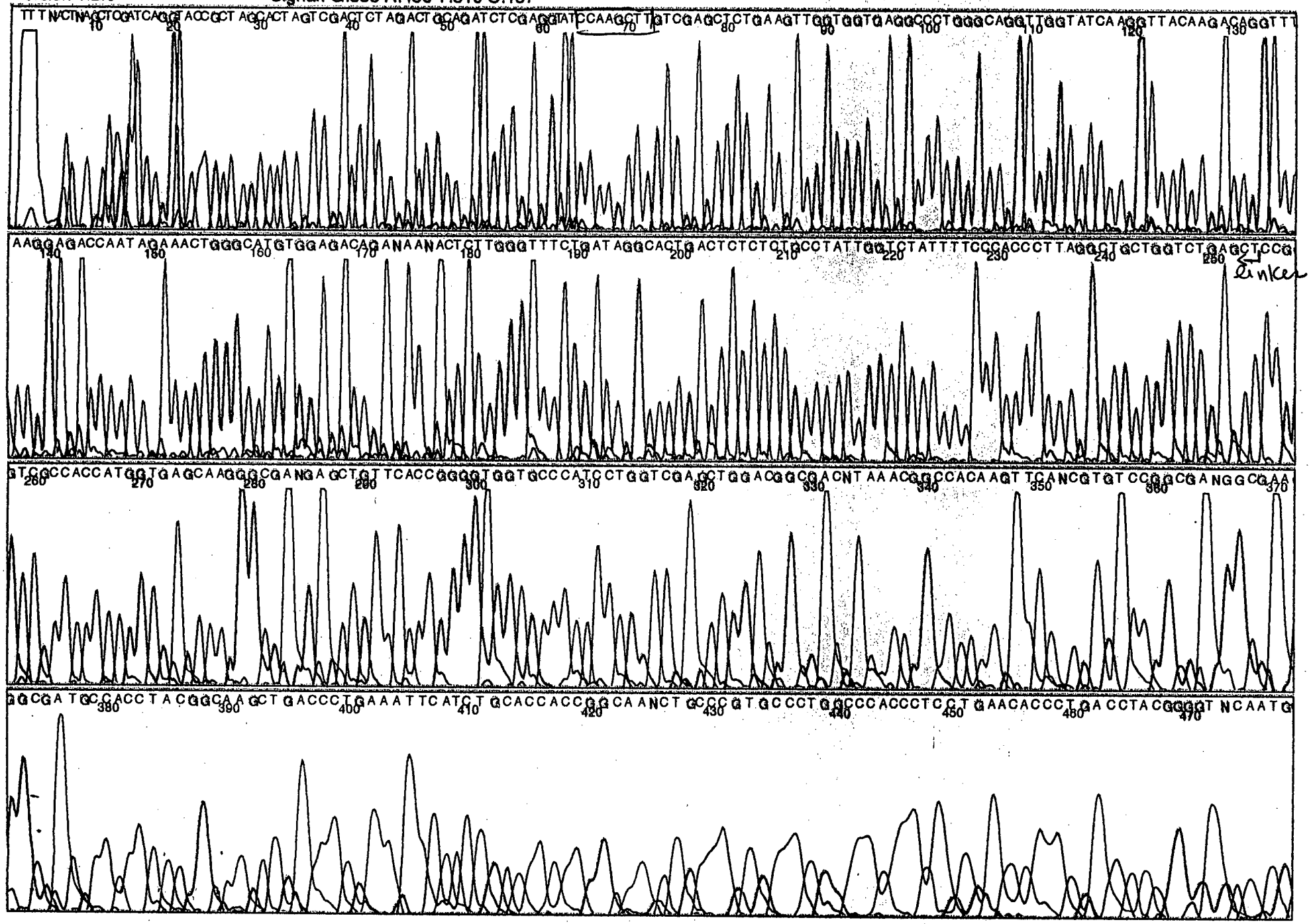
Applied Biosystems
Model 373A
Version 1.2.0

Sample 18.1
DyeTerminator{AnyPrimer}
Lane 18
Signal: G:358 A:486 T:319 C:157

Points 69[illegible]o 6184 Base 1: 690
instrume[illegible]907443
FD 2326

Wed, Aug 21, 1996 2:59 PM Page [illegible] of 2
X: 0 to 6631 Y: 0 to 1200
Spacing: 10.82

for.



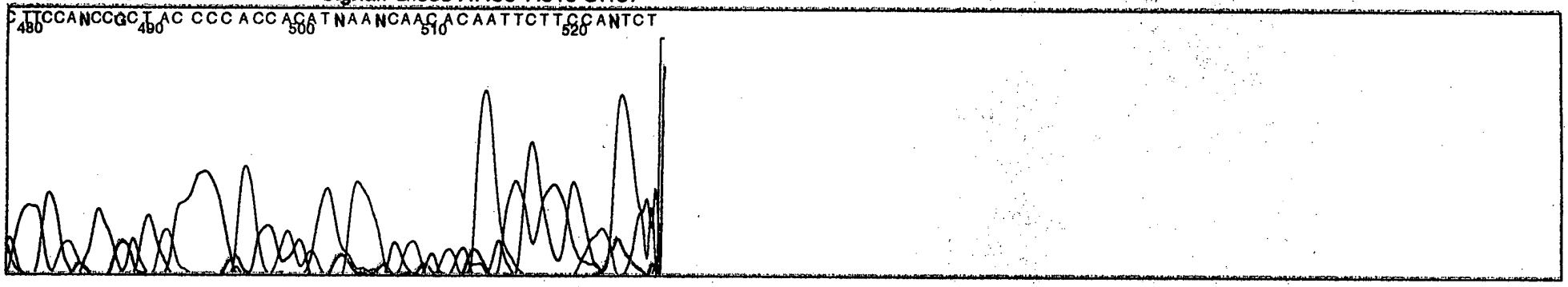
linker

Apple Biosystems
Model 373A
Version 1.2.0

Sample 18.1
DyeTerminator{AnyPrimer}
Lane 18
Signal: G:358 A:486 T:319 C:157

Points 6[illegible] 6184 Base 1: 690
Instrument #907443
FD 2326

Wed, Aug 21, 1996 2:59 PM
X: 0 to 6631 Y: 0 to 1200
Spacing: 10.82



8-15-96

* Maike's frag. → used 50ng vector
~~used 1 µl insert~~ 3 µl insert

* ~~-~~482 rgls promoter (rat glucagon)

* pEGFP-N2 = dbl digest only w/ pos.

carrier DNA: digest; when ppt. w/ EtOH add 1 µl tRNA then continue

* plasmid prep → dble CsCl (2x) of pEGFP-N2
* also inculture & do 20-tip of pEGFP-N2
* always leave 1 µl of DNA from prep.
* transform 1 µl DNA for a plate
~~* for~~

* for BamHI / HindIII digest use Buffer B; can phenol/chloroform purify or gel purify

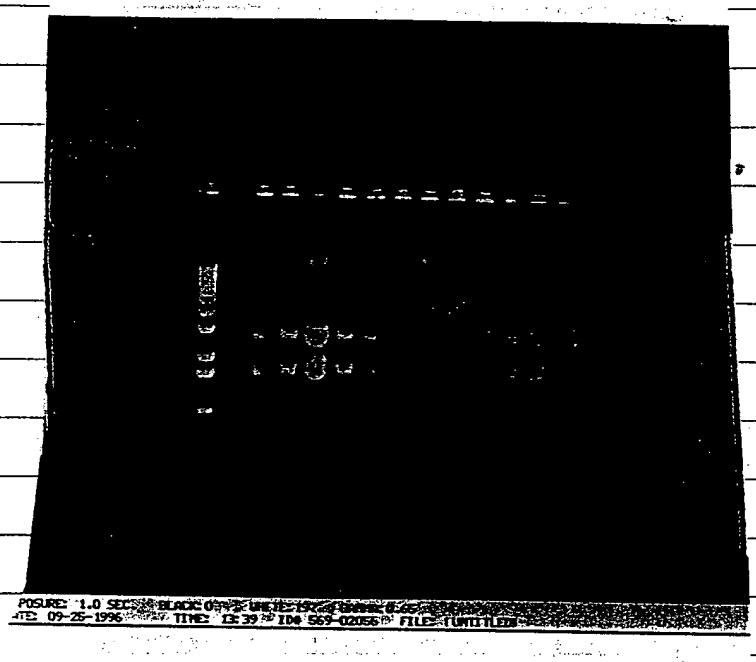
* intron - inc. exp. level & Thus sens. of system

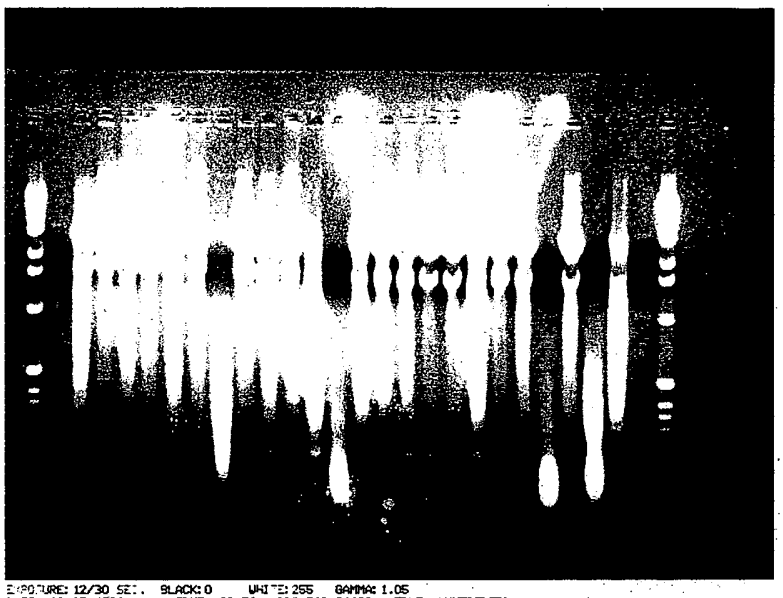
*

8.24-96

* minis pEGFP-N2 -482 rglc Z- element w/ EcoRI overnight

pick minis for pEGFPN2-482 rglc Z element 9-25-9





EXPOSURE: 12/30 SEC. BLACK: 0 WHITE: 255 GAMMA: 1.05
DATE: 08-25-1996 TIME: 09:56 ID# S69-01698 FILE: (UNTITLED)